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THE ANATOMY OF THE REPTILIAN HEART*

Part I. *Varanus monitor* (Linné)

BY PRAHLAD NARAIN MATHUR, PH.D.

Received December 20, 1943
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I. Introduction

THE reptilian heart forms a transitional stage of great evolutionary significance. The reduction of the sinus venosus, the complete separation of the auricles by a septum which reaches up to the atrio-ventricular aperture and is unperforated in the adult, the division of the ventricle by a complete or incomplete septum, the absence of the conus arteriosus, and the consequent separate origin of the arterial trunks from the lumen of the ventricle—all these features, showing a distinct advance over the amphibian heart and a decided approach towards the condition found in the higher Amniota, have been generally recognised (Gegenbaur, 1898–1900; Wiedersheim, 1907; Nierstrasz, 1927; Kingsley, 1926, Goodrich, 1930; Benninghoff, 1932, etc.). Our knowledge, however, of the comparative minute anatomy of the heart within the limits of Reptilia itself is far from adequate, and so far nobody has studied this organ minutely by serial sections in representatives of all the principal subdivisions There is much disparity of observations and opinion even about such a conspicuous structure as the muscular

* Part of a thesis approved for the Ph.D. Degree in Agra University.

ridge ('Muskelleiste') and a detailed examination of the disposition of the various apertures and their valves, of the chambers in the ventricle, and of the exact mode of origin of the trunks, cannot but be welcome.

In the first part of the present series, the heart of *Varanus monitor* (Linn.), a representative of Sauria, is described in detail in order to add to our knowledge of the minute anatomy of this organ in the Reptilia, to serve as a basis for work on other reptiles, and to supply the needs of Indian students who study this lizard as part of their zoological syllabus Subsequent parts will deal with representatives of other orders, as well as with the *modus operandi* of the reptilian heart.

2 *Historical Résumé*

Although certain contributions were made by earlier workers (*e.g.*, Bojanus, 1819–1821; Bischoff, 1836; Duvernoy, 1838, Vrolik, 1841; Hentz, 1825; Panizza, etc.), the foundation of our knowledge of the reptilian heart was laid during the middle of the last century by the pioneer researches of three German morphologists—Brucke, Rathke, and Fritsch The first made an extensive, as well as detailed study of it in numerous reptiles (1852) and dealt with the mechanism of circulation (1850) in the Testudinata. The second investigated the aortic roots in Sauria (1857), as well as the development of the heart in snakes (1839) and turtles (1848). The third (1869) devoted himself to a comparative anatomy of the heart in a great many representatives of what he called the 'Amphibia', but what included both the Amphibia and the Reptilia of the modern classification.

The lead given by these workers was followed by a host of others who, in a majority of cases, kept up the high standard set before them. Thus gradually in less than a century a great mass of knowledge accumulated, dealing with the reptilian heart from diverse points of view—morphological, developmental, histological and physiological.

(*a*) *Comparative Anatomy and Development.*—Owen (1866) gave a detailed, though generalised, account of the heart in various subdivisions of the Reptilia, based partly on the researches of previous authors, but mainly on his own observations, Huxley (1871) distinguished and described three forms of heart in the Province *Sauropsida* (= Reptilia and Aves), instituted by him; *viz.*, the first form, found in the *Testudinata*, *Sauria* and *Serpentes*; the second in the *Loricata*, and the third in the *Aves*. Sabatier (1873–74) surveyed the heart, central circulation, and transformation of the aortic system in the vertebrate series. Hochstetter (1893 and 1906) traced its development in *Tropidonotus* and *Lacerta*. Langer (1894) studied the development of the *truncus* and *bulbus cordis* in Amphibia, Reptilia, Aves and

Mammalia, and pointed out the manner in which this region becomes subdivided during ontogeny. Gegenbaur (1901) gave a masterly account of the comparative anatomy of the vertebrate heart. Greil (1903) made a study of the comparative anatomy and development of the heart and truncus arteriosus in vertebrates. Goodrich (1916 and 1919) pointed out the phylogenetic importance of the position of the reptilian *septum ventriculorum* in relation to the openings of the arterial trunks, recapitulated the structure and functioning of the reptilian heart (1930), and pointed out the manner in which the bulbus cordis gets spirally subdivided in the vertebrate series (1930) O'Donoghue described the hearts of *Tropidonotus natrix* (1912) *Dermochelys coriacea* (1918) and *Sphenodon punctatus* (1920), sketched successive sections through the bases of the arterial trunks in *Sphenodon* (1920), and criticised Goodrich's views on the basis of certain observations on the *septum ventriculorum* in various reptiles (1918). Hesse (1921) dealt with the weight of the heart in vertebrates, having previously (1908) noted the relation between its size and efficiency. Barry (1921) investigated the path of conduction between the auricles and the ventricle in the amphibian and reptilian hearts. Rau (1924) studied the hearts of *Tiliqua scincoides* and *Eunectes murinus* in detail and tried to throw light on the points of disagreement between O'Donoghue (1918) and Goodrich (1916 and 1919). Benninghoff scrutinised the architecture of the cardiac muscles (1923 and 1931), and gave an excellent comprehensive résumé of the knowledge about the reptilian and other hearts (1931). Nierstrasz (1927) gave a lucid exposition of the salient features of the reptilian heart in order to point out its evolutionary status in the vertebrate series. Bremer (1928) tried to explain the mechanical reasons for the connection of the left aorta of reptiles with the right ventricle. Skramlik (1932) studied the relative position of the sinu-atrial aperture in reptiles, and Mahendra (1942) established four regions in the saurian ventricle on the basis of serial sections of *Hemidactylus flaviviridis* Rüppel.

(*b*) *The Heart in Sauria.*—The heart Saurian has not attracted many workers in the present century Imchanitzky (1909) scrutinised the question of nervous co-ordination of the auricles and ventricle in it. Rau (1924) studied it in *Tiliqua scincoides* by means of dissections, transverse sections, and a wax reconstruction, and compared it with that of *Eunectes murinus*. Vorstman (1933) pointed out the resemblance of the ventricular septa of *Varanus komodœnsis* to those in snakes. Bhatia (1929) dealt with the heart of *Uromastix hardwickii* and gave valuable sketches of transverse sections passing through the origin of the arterial trunks, and Mahendra (1942) studied it in *Hemidactylus flaviviridis* minutely by the reconstruction method.

3. Technique

The general structure of the heart was studied in dissections under the binocular microscope; coloured bristles, passed through the various apertures, proved valuable aids.

Minute anatomy was studied in serial sections, 10 μ thick, both transverse and horizontal-longitudinal, prepared according to the paraffin embedding process. Carnoy's fluid and Bouin's picro-formol were used as fixatives, and Grenacher's Borax carmin, Acid fuchsin, Ehrlich's Acid Hæmatoxylin (counterstained with 1 per cent. alcoholic eosin), and Mallory's triple stain were used as stains

Diagrams were made at first by means of Abbe's camera lucida with Reichert's eyepiece II (5×) and objective O (3 2×), but as the work progressed it was found more convenient to sketch the sections by projecting their images on the drawing paper by means of a Zeiss-Ikon Epidiascope fitted up with a micro-attachment.

4 General

The heart of *Varanus monitor* (Linn.) lies in the mid-ventral line (Fig. 1) definitely behind the axillary region, partly embedded in a deep notch in

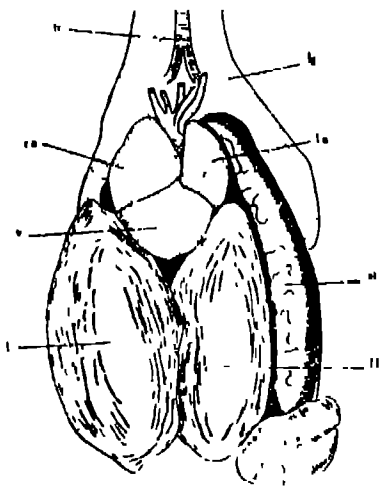


FIG 1 Ventral view of the heart of *Varanus monitor*, *in situ* in the pleuroperitonial cavity. *l a*, the left auricle, *lg*, lung, *l l*, left lobe of the liver, *r a*, the right auricle, *r l*, right lobe of the liver; *st*, stomach; *tr*, trachea, *v.*, ventricle

the anterior border of the right and left lobes of the liver, and bounded on its right side by the right lung and on its left by the cardiac part of the

stomach. Such a position is distinctly posterior to that of the heart of *Uromastix hardwickii* Gray, *Calotes versicolor* (Daudin), *Mabuya dissimilis* (Hallowell) and *Riopa punctata* (Gmelin), and is probably to be correlated with its higher grade of organisation. As Rathke (1857) pointed out, the farther back is the heart situated in the pleuroperitoneal cavity, the more highly organised is the reptile.

The heart is enclosed in a fairly tough serous membrane, the pericardium, and approaches the testudinate heart in its form, being almost as broad as long. In a full grown individual it measures 4·2 cm. in length and slightly less in width. It consists of a sinus venosus, two auricles and a ventricle. The right auricle is considerably larger than the left; the ventricle, unlike that in other lizards, is broader than long and distinctly rounded behind, and there is no trace of a *gubernaculum cordis*.

5. *Sinus Venosus*

As pointed out by Wiedersheim (1907), Kingsley (1926), Goodrich (1930) and other authors on the comparative anatomy of Vertebrates, the *sinus venosus* in reptiles, although much reduced is generally distinct internally. In the Sauria it resembles that of *Sphenodon punctatus* in its general form and arrangement. A description of it has recently been given by Rau (1924) in *Tiliqua scincoides*, by Bhatia (1929) in *Uromastix hardwickii* and by Mahendra (1942) in *Hemidactylus flaviviridis*. In *Tiliqua scincoides*, although not clearly marked off externally, it lies attached to the right auricle a little to the left. In *Uromastix hardwickii*, it is the " largest " (?) chamber of the heart, lying transversely above the auricles and marked out externally into a large right and a small left portion by a slight constriction in the middle. In *Hemidactylus flaviviridis* there is no such constriction, but the dorsal wall of the sinus venosus is strongly fluted in order to accommodate the trachea which lies closely adpressed to this structure

The *sinus venosus* in *Varanus monitor* (Fig. 2) is scarcely distinguished externally from the bases of the venæ cavæ except for the contractility observable in this part in a freshly killed specimen. The left precaval vein enters the anterior border of the pericardium distinctly on the left side and runs obliquely backwards on the roof of the left auricle towards the middle of the *coronary sulcus* where it opens into the sinus venosus. The right precaval vein runs almost straight backwards on the dorsal surface of the right auricle, while the postcaval vein, after entering the pericardium, passes directly forwards along the right side of the ventricle to become confluent with the base of the right precaval and discharges there into the sinus venosus.

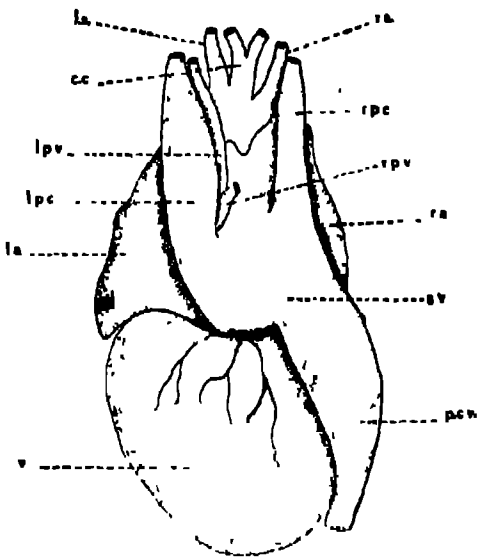


FIG 2 Dorsal view of the heart in *Varanus monitor*

c.c the common carotid artery, *l p c*, the left precaval vein, *l p v*., the left pulmonary vein, *l s*, the left systemic artery; *p c v*, the post-caval vein, *r p c*, the right precaval vein, *r p v*, the right pulmonary vein; *r s*, the right systemic artery, *s v*, the sinus venosus; Other abbreviations as in the previous figure

Such a disposition of the venæ cavæ is responsible for a curious asymmetry of the sinus venosus, so that its greater part lies rather towards the right of the middle line of the heart. An annular constriction, situated close to the base of the left precaval vein and distinct not only in a superficial view but also in dissections of this region, divides the sinus venosus into two divisions: a small right one, confluent with the base of the left precaval vein, and a large right one, receiving the right precaval and postcaval veins. While the sinus venosus is barely distinguishable externally, it is found, on removing the dorsal wall of the heart, that this chamber is delimited from the bases of the constituent venæ cavæ as a shallow, ovoidal depression in the roof of the right auricle, which bears the *sinu-atrial aperture* and *valves* (Fig. 3). Its disposition indicates that it is in a fairly advanced state of reduction.

The *sinu-atrial aperture*, a large slit-like opening, lies obliquely inclined from a transverse direction in such a manner that its right end is situated craniad to its left one. It extends almost the whole extent of the breadth of the sinus from the right portion of the latter chamber across the mesial constriction even to a small extent into its left portion.

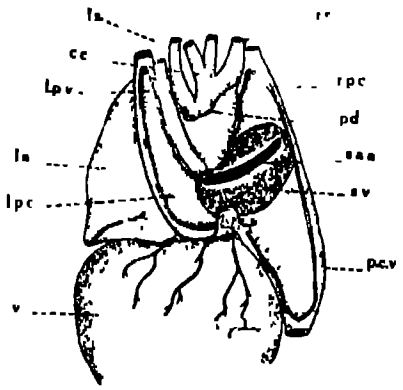


FIG 3 Sinus venosus of *Varanus monitor* with the dorsal wall removed to show the sinu-atrial aperture and valves

pd, the pericardial attachment; *s a a*, the sinu-atrial aperture, other abbreviations as in previous figures

In *Uromastix hardwickii*, Bhatia (1929) observed that the sinu-atrial valves are absent but that the lips of the sinu-atrial aperture are thick and muscular, serving thereby to keep the aperture ordinarily closed In *Varanus monitor* the *sinu-atrial valves* are definitely present They are developed on the cranial and caudal edges of the aperture and lie directed like a pair of almost parallel flaps obliquely downwards into the lumen of the right auricle

As the minute structure of the sinu-atrial valves in the Sauria has not been hitherto described, I have studied it carefully in a series of horizontal longitudinal sections. The caudal valve, when seen from the dorsal aspect (Fig. 3), overlaps the cranial one towards the left end of the sinu-atrial aperture while the two valves lie at the same level at the right end of the aperture and are united to form a solid strand of tissue attached to the right wall of the auricle. We might call this solid strand of tissue the *suspending ligament* (Fig. 4, B) as it serves to support and suspend the sinu-atrial valves.

On the dorsal border of the suspending ligament (Fig. 4, A) is a narrow canal (the *sinu-atrial channel*), not described so far, which leads from the dorsolaterally situated sinus venosus into the lumen of the auricle. The suspending ligament is produced at its free left end into the sinu-atrial valves, hanging freely into the auricular space There are no tendinous cords attached to the free ends of the valves. The suspending ligament is rather obliquely directed and its ventral part (Fig. 4, C) which lies attached to the right wall of the auricle shows neither the valvular growths at its free margin, nor the sinu-atrial channel.

Such a disposition of the sinu-atrial valves makes it clear why the blood can pass from the sinus venosus into the right auricle, but not *vice versa*. When the sinus venosus contracts, the increased pressure on the blood forces it into the sinu-atrial channel, and drives the blood into the lumen of the auricle. In auricular systole, however, the pressure of the blood is outside the sinu-atrial valves and channel. It, therefore, closes them so that the blood cannot go back into the sinus venosus. The two valves are not equal in size (Figs 4 and 5), but the free border of one valve projects beyond

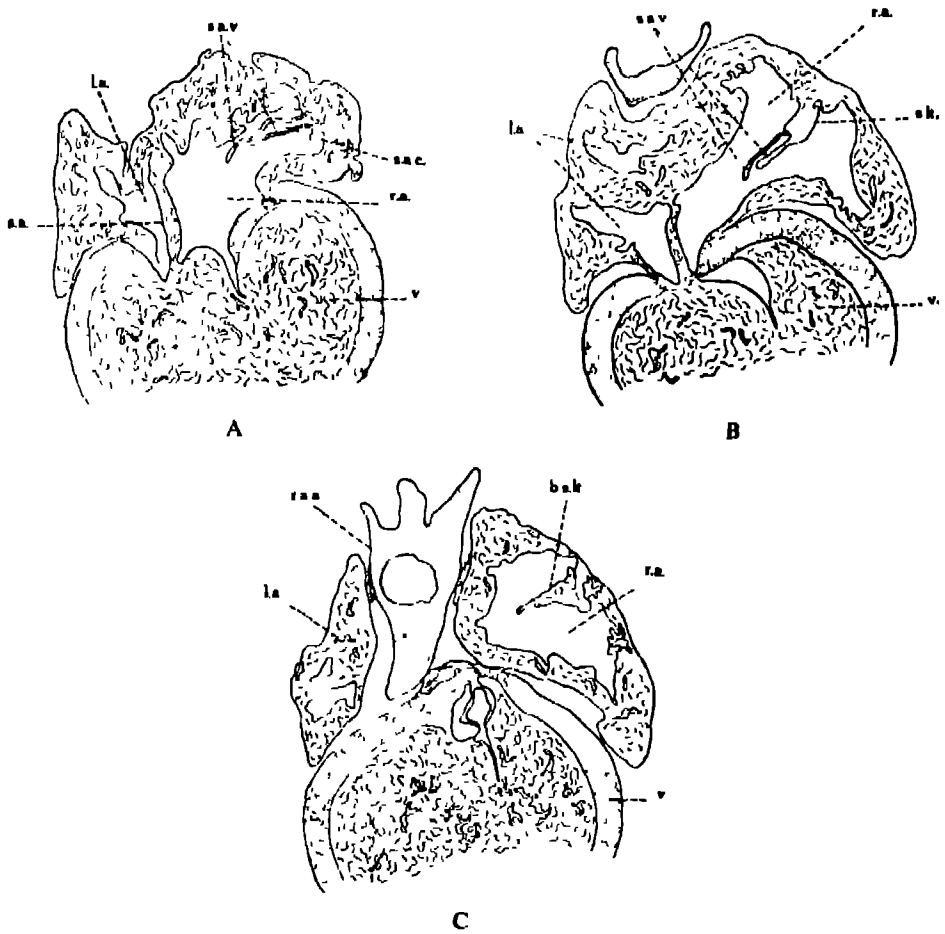


Fig 4 Horizontal longitudinal sections through the sinus venosus and auricle of *Varanus monitor* : (A) At the level of the sinu-atrial channel (B) immediately ventral to it ; and (C) through the lower part of the base of the suspending ligament (× 5)

b.s lt, the base of the suspending ligament, *r a a*, the roof (dorsal wall) of the aortic arches; *s.a*, septum atriorum *s a c*, the sinu-atrial channel; *s.a v.*, the sinu-atrial valve, *s lt*, the suspending ligament, other abbreviations as in previous figures.

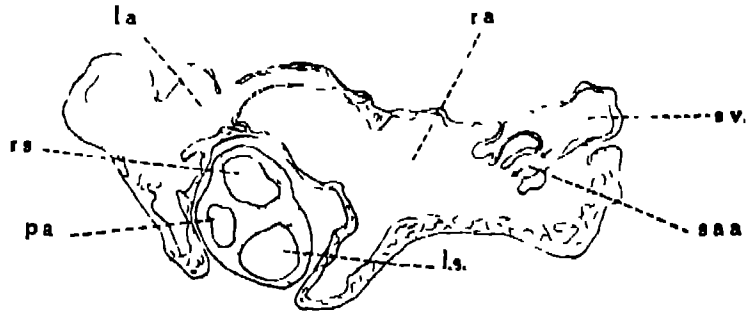


FIG 5 Transverse section through the auricular region of *Varanus monitor*, showing the sinu-atrial aperture and valves (× 7)

p a, the pulmonary trunk. Other abbreviations as in previous figures

that of the other This projecting border, during auricular systole, would bend over the sinu-atrial aperture and the other valve, and thus serve as an additional aid in the closure of the opening

As in *Sphenodon punctatus* (O'Donoghue, 1920), there is no ridge (*tuberculum intervenosum*), separating the opening of right precaval from that of the post-caval Nor is there any septum (*septum sinu-venosi*) between the openings of the left precaval and post-caval veins on the one hand and that of the right precaval on the other (*cf.* Goodrich, 1930, p 556).

6. *Atrium Dextrum*

The right auricle (*atrium dextrum*) is larger than the left—a disparity in sizes which has been noted frequently in Lizards (*e g*, Rau, 1924 in *Tiliqua scincoides*; Bhatia, 1929 in *Uromastix hardwickii*; Mahendra, 1942 in *Hemidactylus flaviviridis*, etc.); in *Sphenodon punctatus* (O'Donoghue, 1920), in Testudinata (Burne, 1905 and O'Donoghue, 1918 in *Dermochlys coriacea*), and in Loricata (Reese, 1915 in *Alligator mississippiensis*) It goes without saying that the differences in size and disposition of the auricle, as well as that in the position of their openings into the ventricle, are significant factors bearing on the mode of action of the ventricle.

O'Donoghue (1920) observed a small sac-like diverticulum at the antero-dorsal edge of the right auricle in *Sphenodon punctatus*, and Bhatia (1929) described a similar structure in the Lizard, *Uromastix hardwickii*. There is no such structure in *Varanus monitor*.

The internal lining of the auricular wall is raised up into numerous strands of *trabeculæ*, composed of muscular fibres and dividing the peripheral part of the lumen into a series of irregular spaces which communicate with one other. These strands have been compared to the *musculi pectinati*

of higher vertebrates (O'Donoghue, 1920 and Bhatia, 1929). While small trabecular growths can be made out almost all over the internal surface of the auricular wall, two obliquely disposed muscular strands (Fig 6) stand out

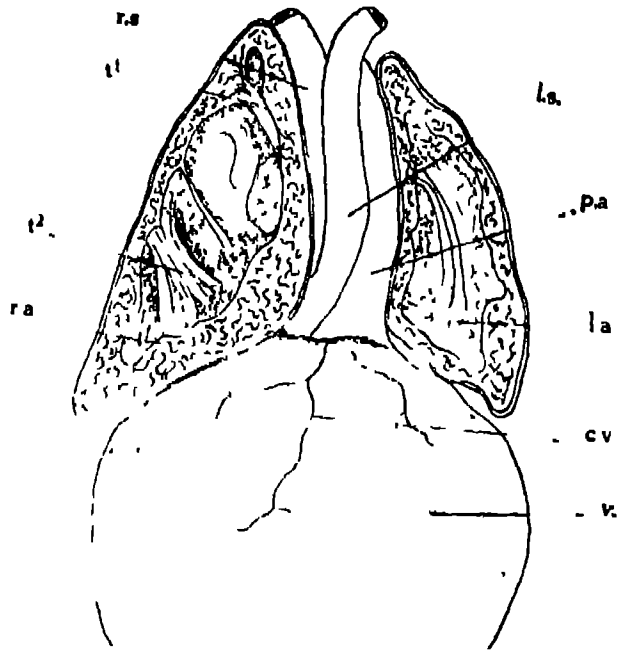


FIG 6. The auricles of *Varanus monitor* dissected from the ventral side to show their internal structure (× 5)

c v, the coronary vein; t^1 and t^2, the muscular strands, other abbreviations as in previous figures

conspicuously when the right auricle is dissected. One of these (t^1) extends from near the anterior end of the inner wall of the auricle to the anterior region of the outer wall of this chamber where it branches into a number of small muscular strands. The other (t^2) is situated near the posterior conical part of the auricle. The purpose of these strands seems to be to contract the anterior and posterior tapering regions of the auricle and to force the blood from them towards the atrio-ventricular aperture.

The *sinu-atrial aperture* is situated in the dorsal wall of the right auricle almost midway between its anterior and posterior ends. When seen from the ventral side it is found to extend obliquely backwards from the outer border of the auricle to the inner. It is bounded by the inwardly-hanging cranial and caudal *sinu-atrial* valves, which have already been described in the section on *sinus venosus*.

The right auricle as in other Reptiles is separated from the left by an unperforated *interauricular septum.* In *Tiliqua scincoides* Rau (1934) found that this septum "is not quite vertically disposed but is oblique and has its concave surface towards the cavity of the right auricle. It is not quite tensely stretched". The septum in *Varanus monitor,* when seen in horizontal-longitudinal sections (Fig. 4, A) extends straight backwards from its anterior end to its posterior. In transverse sections (Fig. 7), however, it clearly runs

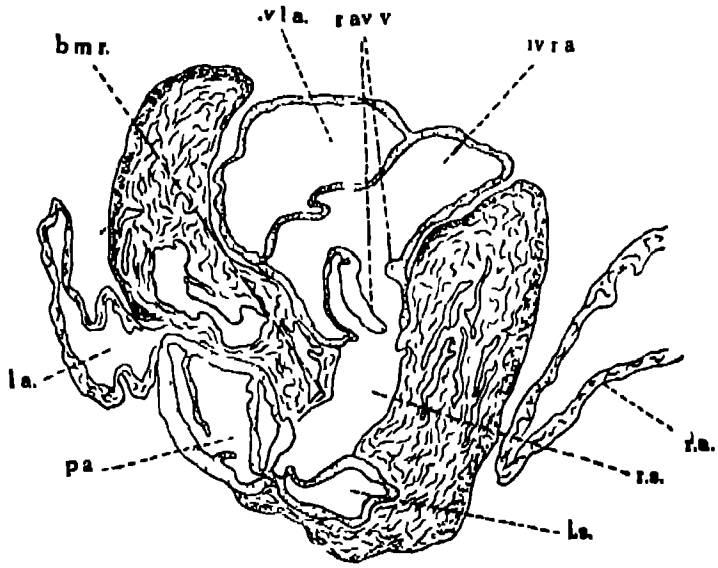


FIG 7 Transverse section through the posterior part of the auricles and the anterior part of the ventricle, to show the atrial septum and the right atrio-ventricular aperture (× 10)

b m r, the base of the muscular ridge, *iv l a*, atrium sinistrum intraventriculare, *iv r a*, atrium dextrum intraventriculare, *r av v*, the right atrio-ventricular valve, other abbreviations as in previous figures.

from the right side above obliquely downwards towards the left I cannot attach any importance to the concavities or convexities observable in its surface when it is limp, as these disappear when the auricular lumen is turgid with blood and are not always caused definitely at the same place when the auricles contract.

The posterior inner region of the right auricle is driven backwards and rather downwards into the lumen of the ventricle to form what may be called the *atrium dextrum intraventriculare* Its wall, even though it lies here inside the ventricle, is distinctly atrial in character (Fig. 7); further posteriorly, it gets incorporated into the ventricle in the form of a truncated funnel. Thus the *atrioventricular aperture* lies an appreciable distance inside

the ventricle itself. A scrutiny of serial transverse and longitudinal sections leaves no doubt that the right and the left atrio-ventricular apertures are not situated side by side at the same level, but that the right aperture lies far craniad to the left one (*cf.* Fig. 9, B with Fig. 7) The latter opens downwards into a chamber of the ventricle called by me the *cavum dextrum* (*vide infra*).

As for the number of the atrio-ventricular valves there is considerable difference of opinion amongst previous workers. Some think that there are four such valves while others hold that there are only two.

According to Nierstrasz (1927, p. 672) " the *septum atriorum* in the Amphibia reaches almost up to the ostium atrio-ventriculare and there are at its free edge two valves fused together at their base, one dorsal and the other ventral, which, as it were, are divided into two parts by the septum, so that really four half-valves seem to be present, two right and two left. In the Reptilia the septum becomes longer, whereby the two said valves are actually divided into two parts, so that there appear two valves united at the base to the right and two to the left of the septum. These are membranous structures attached to the wall of the septum In the *Loricata*, moreover, a muscular valve occurs on the lateral wall of the right *ostium atrio-ventriculare*".

Goodrich (1930, p. 557), however, observes that in Reptiles " . along the free edge of this interauricular septum crossing the atrio-ventricular opening are attached two membranous valves projecting into the ventricular opening." He obviously means one valve in the right atrio-ventricular opening and the other in the left· a statement which is confirmed by Rau's observation on *Tiliqua scincoides* that " each auriculo-ventricular opening is guarded by a single valve " (1924, p. 62).

In *Varanus monitor*, however, there are definitely two valves in each atrio-ventricular opening The opening of the *atrium dextrum intraventriculare* into *the cavum dextrum* is guarded by a fairly large left valve and a small right one (Fig. 9, D). The left valve which corresponds to the *septal* (*medial*) *auriculo-ventricular* valve of Goodrich (1919) extends somewhat farther than the right one cranially, while caudally the two valves get fused together so as to separate the posterior extension of the *atrium dextrum intraventriculare* completely from the *cavum dextrum.* Contrary to what has been hitherto said about the basal attachment of the left (septal) valve (Goodrich, 1930 and Nierstrasz, 1927) this valve in *Varanus monitor* is attached anteriorly not to the base of the interauricular septum, but a little away from it to the ventral wall of the *atrium dextrum intraventriculare*

(Fig. 7). When traced posteriorly, however, it is seen to approach towards the base of the septum and finally to be attached to it.

There are no tendinous cords attached to the free ends of the atrio-ventricular valves. Their shape and disposition, however, indicate that a pressure from the side of the *atrium dextrum* would open them towards the *cavum dextrum*, but when pressed in the reverse direction they would knock against each other and block the passage.

7. *Atrium Sinistrum*

The left auricle (*atrium sinistrum*) resembles the right one in its general structure and appearance, but is definitely much smaller. Its wall is built of the same kind of tissue as that of the right auricle, and it is driven backwards, like the right auricle, into the lumen of the ventricle as the *atrium sinistrum intraventriculare* (Fig. 7) It receives aerated blood through the common pulmonary vein

The left pulmonary vein (Fig 2) enters the antero-dorsal border of the pericardial cavity adjacent and parallel to the left anterior vena cava and extends backwards on the roof of the left auricle just to the right of this vein. Almost midway between the anterior and posterior ends of the left auricle, it receives the right pulmonary vein coming towards it in an obliquely backward direction from the right antero-dorsal border of the pericardial cavity. The common pulmonary vein, thus formed, runs transversely towards the left ventrally to the left anterior vena cava, and opens into a prominent crypt in the dorsal wall of the left auricle near the interauricular septum.

According to Hoffmann (1890), the opening of the pulmonary vein into the left auricle is always devoid of valves in Reptiles—an opinion, confirmed by Rau (1924) in *Tiliqua scincoides* and by O'Donoghue (1920) in *Sphenodon punctatus*. In *Uromastix hardwickii*, however, Bhatia (1929) describes a valve which is formed as a lip-like outgrowth from the dorsal wall of the auricle. In *Varanus monitor*, the crypt containing the pulmonary opening is fairly long and it possesses a flap-like outgrowth on one side.

A very important feature in connection with the left auricle in *Varanus monitor* concerns the position of its opening into the ventricle. Serial sections leave no doubt that the opening is far posterior to that of the right atrium (Fig. 7), leads into the cavum dorsale and lies in the posteriormost region of the muscular ridge (Fig. 9, B). It is guarded by a pair of transverse valves.

8. *Ventriculus*

(i) *The Regions.*—Mahendra (1942) recognises four successive regions in the ventricle of *Hemidactylus flaviviridis* Rüppel, passing gradually and insensibly into each other: (*a*) the *apical region*, (*b*) *the region of the muscular ridge* (*Muskelleiste*), (*c*) the *region of the auricular apertures* and (*d*) the *region of the origin of the aortic trunks.* Although these regions may be variously modified in different reptiles and may even shift in relation to each other so as to be more or less co-extensive the recognition of them is a distinct advance in our knowledge of the minute anatomy of the ventricle. It serves not only as a foundation for comparing the structure of the ventricle in the various orders of Reptiles, but also for the elucidation of the rôle of this chamber in the distribution[1] of blood to the arterial trunks.

(ii) *The Apical Region.*—In *Hemidactylus flaviviridis* Rüppel the the apical region, although characterized by the presence of dorsoventrally directed trabeculæ and the consequent breaking up of the space inside into numerous small irregular lacunæ, is not divided into definite chambers. In *Varanus monitor*, however, I find that this region (Fig. 8 A) is distinctly divided into two portions by a septum (*the apical horizontal septum*) composed of transverse muscular fibres: a dorsal portion (*the dorsal apical space or cavum apicis dorsale*) occupying the main bulk of the cavity and traversed by dorso-ventrally directed trabeculæ; and a ventral, narrower portion (*the ventral apical space* or *cavum apicis ventrale*) possessing mostly transverse trabeculæ and rather large spaces.

(iii) *The Region of the Muscular Ridge.*—The muscular ridge (*muskelleiste*) is a prominent structure in the saurian ventricle, noted by Greil (1903), Goodrich (1916, 1919 and 1930), O'Donoghue (1918), Rau (1924), Mahendra (1942) and others, but it was erroneously identified as the *septum ventriculorum* by Goodrich (1916), O'Donoghue, (1918) and Rau (1924). As mentioned by Mahendra (1942) in a footnote, both Greil's and Hochstetter's work on the development of the heart is against such a determination. Benninghoff (1932, p. 514), says " the formation of the ventricular septum, which has been studied by Greil and Hochstetter, takes place out of elements, whose future significance was already alluded to in lower reptiles. There is one or more than one septum in a few lower reptiles, raised up at the place of separation of the arterial and venosus bloods at the lower end of the atrio-ventricular valves. This septum meets ventrally with the muscular ridge, which furnishes the ventral part of the wall of

[1] The *modus operandi* of the reptilian heart will be discussed by the author in a later paper.

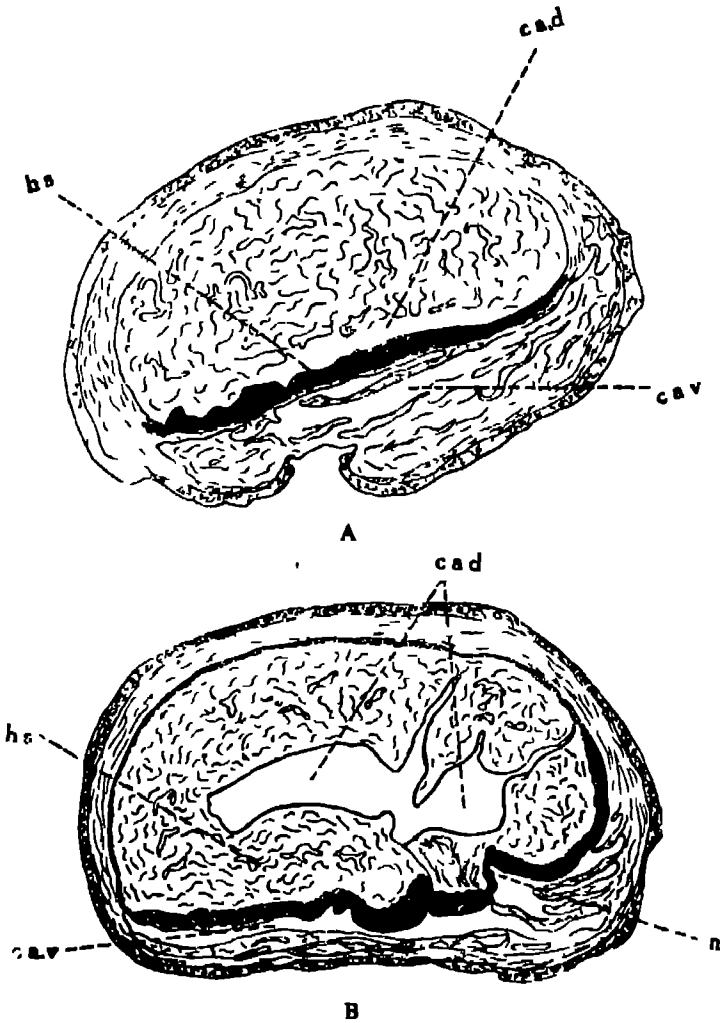


FIG 8. Transverse sections through the anterior (A) and the posterior (B) apical region of the ventricle of *Varanus monitor* (× 10)

c.a d, cavum apicis dorsale, *c.a v.*, cavum apicis ventrale, *h s.*, the horizontal septum, *n*, notch in the horizontal septum just behind the free border of the muscular ridge other abbreviations as in previous figures

the ventricle from this place up to the ventral cortex ('Kortikalis'). Dorsally, according to Greil, the septum is completed by means of a thickening in the trabecular tissue, which extends from the auricular lamella to the cortex (' Kortikalis ') and shifts rather towards the right. Thereby, a small part of the auricular lamella together with the auricular ring is incorporated into the septum." Thus, the fact that the muscular ridge forms only the

ventral part of the *septum ventriculare*, the upper part arising independently of it, is a strong point against regarding it as equivalent to the entire septum.

The *apical horizontal septum* in *Varanus monitor*, when traced forwards (Fig. 8,B), shows at first a fairly large notch on its ventral aspect near the right end. A little anteriorly (Fig 9, A), this notch gives place to a split in the septum so that the ventral apical space communicates with the dorsal. This point marks the boundary between the apical region and the region of the muscular ridge.

O'Donoghue (1918), who examined a large heart of *Varanus salvator*, found that the muscular ridge (called by him erroneously the *septum ventriculorum*) "runs *from the left latero-dorsal wall of the ventricle across towards the right latero-ventral wall*, but is decidedly more dorso-ventral in position than in the Ophidia. As in that group, however, it is incomplete, so that it is only during systole that the ventricular (*sic*) would appear to be completely divided into a right lateral, slightly dorsally situated chamber and a smaller left chamber lying slightly ventrally." Goodrich (1919), however, disagreed with this description and observed that the muscular ridge, "although it may shift somewhat laterally towards the posterior apex of the heart, is *essentially always a ventral septum*, developed in relation to the sulcus inter-ventriculare, which passes back from the bulbo-auricular infolding The septum passes *obliquely dorsalwards from the left ventral wall towards the right, and always tends to separate a left dorso-lateral chamber from a right ventro-lateral chamber* (*cavum pulmonale*)."[2]

Rau (1924), remarked that the muscular ridge in *Tiliqua scincoides*, "springing from the posterior apical wall.. divides the ventricular cavity into a left and right cavity."

A careful study of the orientation of the muscular ridge both in dissections and in series of transverse sections shows that all these descriptions are at variance with facts inasmuch as they strain after interpreting the ridge as a more or less dorsoventrally disposed structure, dividing the cavity of the ventricle into right and left (or dorso-lateral and ventro-lateral) divisions. In *Varanus monitor*, as also in other reptiles, the muscular ridge (Fig 9, C) is horizontally disposed; it is a conspicuous ledge-shaped structure which arises from the lower part of the left wall of the ventricle and has its free border towards its right. It contains numerous minute lacunæ and trabeculæ, and separates a large dorsal space (*cavum dorsale*) from a narrow ventral one (*cavum pulmonale*), the two spaces being continuous with each

[2] I am responsible for italicising the relevant phrases in these quotations.

other over its free border. In the posteriormost part of the muscular ridge the dorsal space is a single continuous cavity (Fig. 9, A), while a little anterior to this point (Fig. 9, B) it is divided by a wide valvular aperture into left and right portions. The left portion, when traced forwards, is seen to be the posteriormost part of the *atrium sinistrum intraventriculare*, and the valvular opening, therefore, must be regarded as the left atrio-ventricular aperture which had shifted far backwards into the lumen of the ventricle. Such an unusual position of the aperture has not been previously described. The right portion of the dorsal space is apparently a part of the cavum dorsale and may be called the *cavum dextrum*

Anterior to the place where the left atrio-ventricular opening is situated (Fig. 9, C), we can distinguish three spaces dorsal to the muscular ridge: (*a*) the *atrium sinistrum intraventriculare* (*iv.l.a.*) completely separated from the space to its right by an oblique dorso-ventral partition, (*b*) the anterior part of the *cavum dextrum* (*c dm*) and (*c*) a small cavity between the dorsal regions of the *atrium sinistrum intraventriculare* and the *cavum dextrum* and proving to be, when traced forwards, the posteriormost extension of the *atrium dextrum intraventriculare* (*iv.r a.*).

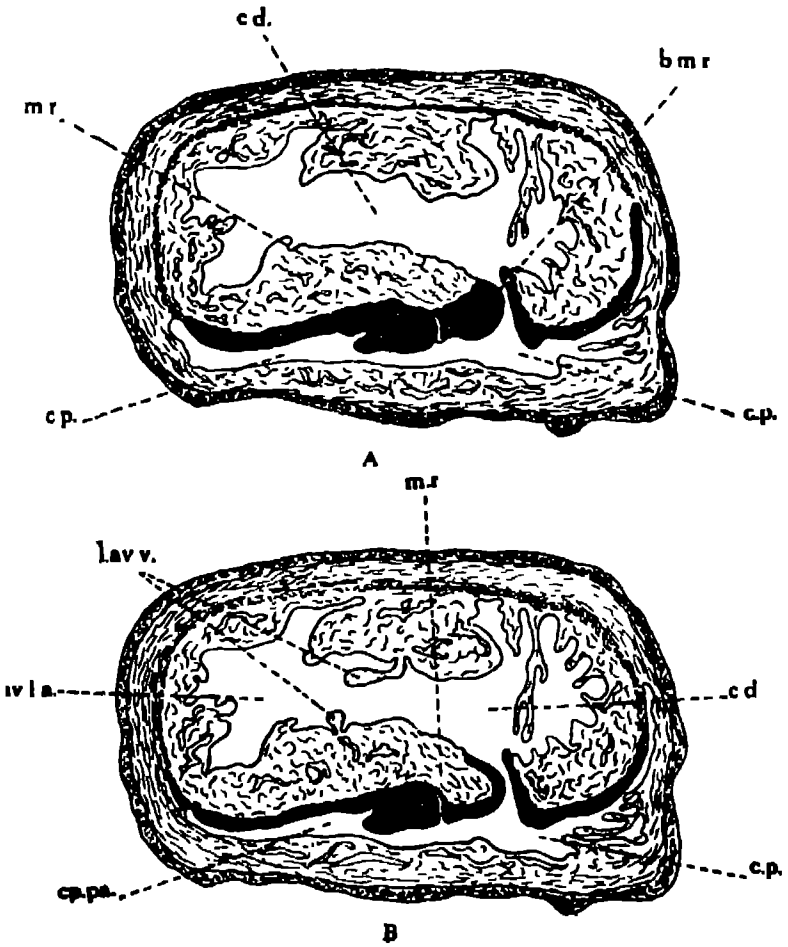
Previous authors (Huxley, Goodrich, O'Donoghue, etc.) distinguished three chambers in the saurian ventricle, generally called the *cavum arteriosum*, the *cavum venosum* and *cavum pulmonale*. While retaining the last term, I have felt it necessary to adopt a new terminology for the rest of the chambers. The terms '*cavum venosum*' and '*cavum arteriosum*' imply that the two chambers designated by them deal with venous and arterial bloods respectively (*cf.* Huxley, 1871, p 265). As will be shown by me in a later paper, this is only partially true. I, therefore, prefer to use the non-committal terms '*cavum dextrum*' and '*cavum sinistrum*' when the space dorsal to the muscular ridge is subdivided; and the term '*cavum dorsale*' when it is not or incompletely divided. Such a procedure, besides avoiding the tendency to prejudge the *modus operandi* of the ventricle on an *a priori* basis serves to bring conformity of designation in this matter to all reptiles including the Loricata.

In *Varanus monitor* the cavum sinistrum is absent, having been ousted by the extraordinarily great backward extension of the atrium sinistrum intraventriculare. Both the cavum dextrum and atrium sinistrum intraventriculare open posteriorly into a single cavity, the cavum dorsale, which extends backwards as the cavum apicis dorsale. The cavum dorsale communicates downwards over the free edge of the muscular ridge with the cavum pulmonale, the latter extending posteriorly as the cavum apicis ventrale.

The left part of the cavum dorsale receives blood directly from the atrium sinistrum intraventriculare, while the right part receives it from the atrium dextrum intraventriculare through the cavum dextrum. The cavum pulmonale is a space leading anteriorly to the base of the pulmonary trunk.

(*iv*) *The Region of the Auricular Apertures.*—The atrio-ventricular openings and their valves have already been described in connection with the two auricles. It may, however, be emphasized that the opening of the *atrium dextrum intraventriculare* (Fig. 7) is situated considerably craniad to that of the *atrium sinistrum intraventriculare* (Fig. 9, B). The former leads into the *cavum dextrum* and the latter into the *cavum dorsale*

(*v*) *The Region of Origin of the Arterial Trunks.*—The exact mode of origin and disposition of the arterial trunks is indispensable for understanding how the contraction (systole) of the reptilian ventricle distributes



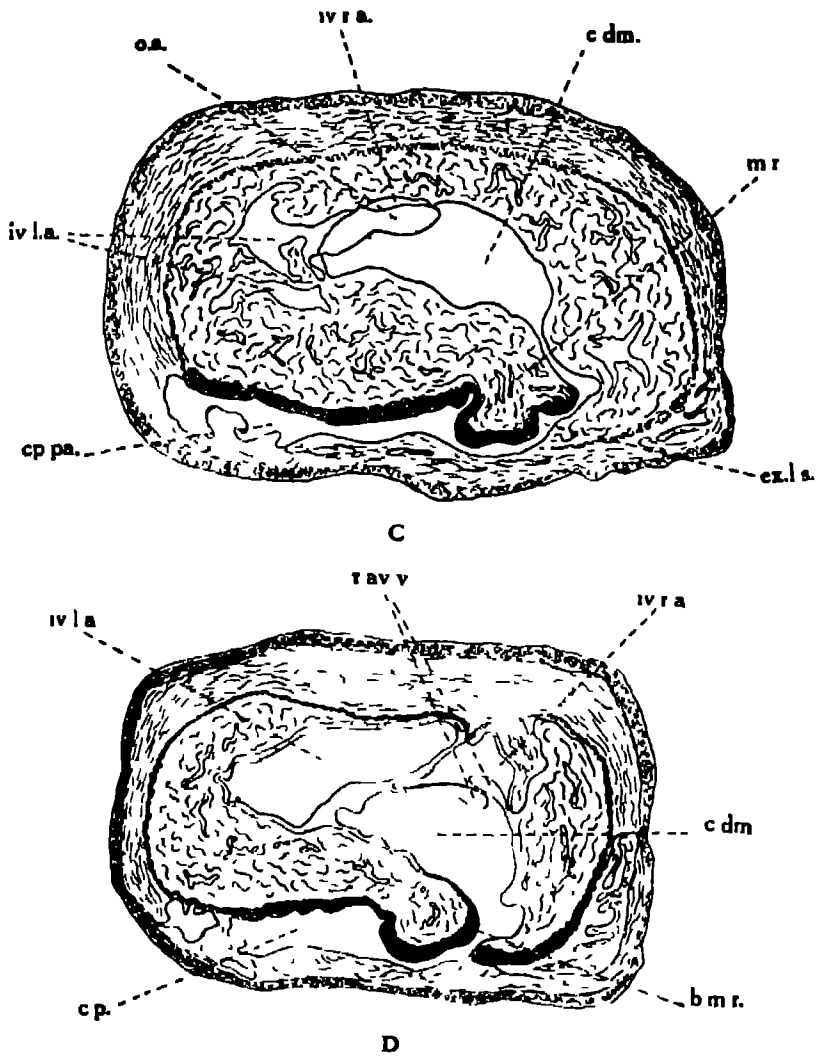


FIG 9 Transverse sections through the region of the muscular ridge in *Varanus monitor* : *A* is the most posterior and *D* the most anterior section (× 10).

c.d, cavum dorsale, *c.dm*, cavum dextrum · *c p*, cavum pulmonale : *cp p a*, part of the cavum pulmonale, leading forwards into the base of the pulmonary trunk *ex l s* the posterior extension of that space which leads forwards into the base of the left systemic trunk, *l.a v v.*, the left auriculo-ventricular valve ; *m r*, the muscular ridge ; *o s*, the oblique septum, other abbreviations as in previous figures

the various kinds of blood. Any addition, therefore, to the knowledge of their minute structure and arrangement, as well as to the valves found in them, cannot but be useful. If we exclude Rau's careful investigation

(1924) in *Tiliqua scincoides* and the excellent figures of their bases by Bhatia (1929) in *Uromastix hardwickii*, there are scarcely any previous observations on their relationships as revealed in serial sections. Besides their importance in physiological interpretation, the disposition of the arterial bases, particularly their relationship to the muscular ridge, is of considerable interest in view of the Goodrich-O'Donoghue controversy. In 1916, Goodrich claimed that in all the living Reptilia the *muscular ridge*, called by him (as well as by O'Donoghue) the *intraventricular septum* (*septum ventriculorum*), tends to divide the ventricle " into a left cavity leading to the base of the right systemic arch, and a right cavity leading to the base not only of the pulmonary, but also of the left systemic arch ", and that such a subdivision of the Sauropsidan bulbus down to its very root could lead only to the Avian type, but in no way to the Mammalian In 1918, O'Donoghue contested Goodrich's observations on the basis of a study of twenty representative reptiles and concluded that " the condition in Ophidia and Lacertilia are quite different from what is implied by Goodrich ". According to him " the ventricle in these two groups containing by far the greater number of living species of reptiles, is indeed partially divided into a right and left chamber, but the two systemic arches come off from the right side and the pulmonary arch alone comes off from the left. There is thus a considerable difference in the relation of the septum to the arterial trunks between the Ophidia and Lacertilia on the one hand and the Crocodilia and Chelonia on the other Not only is there a greater twist on the arterial trunks, which leave the top of the heart in relatively the same position, while the pulmonary leaves the ventricle more to the right in the latter, but the septum is actually situated on opposite sides of the pulmonary artery. In Ophidia and Lacertilia it lies between the pulmonary and left systemic, while in Crocodilia and Chelonia, it lies between the pulmonary and right systemico-carotid." In 1919, Goodrich in a rejoinder to O'Donoghue maintained his point of view but supplemented it further with a mass of new details. In 1924, Rau investigated the structure of the heart of *Tiliqua scincoides* in order to check the results of both these authors, and concluded that " the contention of Dr. O'Donoghue in regard to the relation of the interventricular septum to the ventricular wall is unfortunately based on imperfect data and his statement concerning the course of blood flow in Lacertilia and Ophidia is obviously erroneous." While thus supporting Goodrich, Rau did not express any opinion about the relation of the muscular ridge—the crux of the problem which had evoked the controversy.

Before trying to adjudge these conflicting viewpoints, it is desirable to describe accurately the condition in *Varanus monitor*. When we trace

forwards the cavities of the ventricle (Fig. 9, D), we find that the pulmonary trunk (Fig. 10) is the first to be delimited from the surrounding spaces. It lies ventral to the muscular ridge a little towards the left side and is formed by a process of closure of the *cavum pulmonale*. It is clear from a comparison of the sections showing the posteriormost part of its definitive base (Fig. 10, A) with those in which it is not definitely delimited (Fig. 9, D) that during the ventricular systole when the muscular ridge would be firmly applied against the ventricular wall, the pulmonary trunk can receive blood not from the dorsal spaces but only from the *cavum pulmonale* and the *cavum apicis ventrale*.

Immediately anterior to the place where the base of the pulmonary trunk is definitely enclosed, the left systemic trunk (Fig. 10, B) gets delimited to

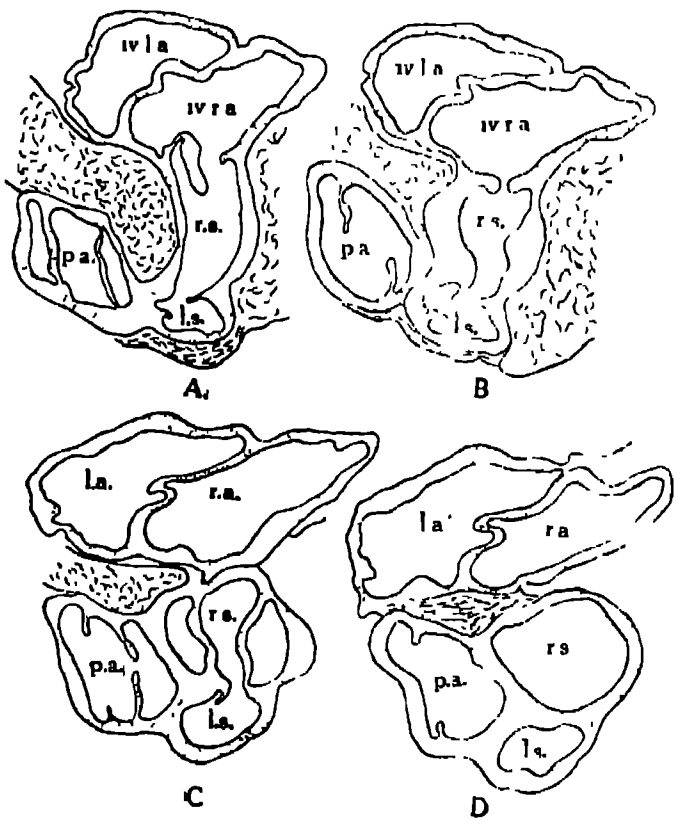


FIG. 10. Transverse section through the base of the arterial trunk in *Varanus monitor*

(A) At the place where the base of the pulmonary trunk arises (B) A little anterior to the section figured in A. (C) Slightly anterior to the section figured in B (D) At the level where all the three arterial trunks are formed. (× 12).

Abbreviations as in previous figures.

the right of the pulmonary trunk Its base lies ventral to the space in which a little anteriorly the right systemic makes its appearance Traced backwards, the left systemic is found to extend as a narrow space at the free border of the muscular ridge between the ridge and the ventricular wall. Evidently, therefore, during the ventricular systole when the ridge is pressed strongly against the wall, this trunk is virtually closed and can receive blood only from its dorsal side through the base of the right systemic where it is open

The right systemic arch is the anteriormost to be enclosed definitively (Fig 10, C) It arises dorsal to the left systemic but it shows many important features. In the first place, its base is continuous posteriorly with the *cavum dextrum*. As the latter lies dorsal to the muscular ridge, the right systemic cannot be shut off from this space during the ventricular systole. Secondly, the right systemic receives blood from the atrium dextrum intraventriculare by a large aperture on its dorsal side Thirdly, the base of this trunk opens downwards into the left systemic arch.

Thus, although the origin of the pulmonary trunk from the space (*cavum pulmonale*) ventral to the muscular ridge agrees with the description of the previous authors, there are some differences worth noting about the other two arches. Goodrich originally (1916) believed that the muscular ridge in reptiles lies between the pulmonary and left systemic arch on the one hand and the right systemic on the other, while O'Donoghue (1918) stated that in Sauria the "left chamber" (= *Cavum pulmonale*) gives off only the pulmonary artery, and both the systemic arches come off from the "large right chamber" (= *the space dorsal to the muscular ridge*).[3] Later (1919), Goodrich modified his position to some extent and remarked that in the Testudinata "the left arch receives most of its blood from the cavum pulmonale", but in the Sauria and Serpentes "by an extra twist upwards of the base of the left systemic arch its ostium comes to lie close to that of the right arch and dorsally to the free edge of the septum, thus receiving more of the arterial blood." Contrary to these views the left systemic trunk in *Varanus monitor* lies neither dorsal nor ventral to the ridge, but at its free border. Thus during sytole when the muscular ridge is adpressed to the opposite wall it would be cut off from the cavum pulmonale, receiving blood only from the dorsal space.

Bhatia (1929) has figured and described the valves in the bases of the aortic trunks in *Uromastix hardwickii* and has compared them with those found in *Sphenodon punctatus* (O'Donoghue, 1920). He finds that "each of

[3] O'Donoghue's schematic representation of the condition in Ophidia and Lacertilia (his Fig 3, C and D), however, show the right systemic trunk situated at the free border of the ridge.

the three trunks is independently provided with a pair of semilunar valves, which are formed from the lining membrane, strengthened by fibrous tissue. They are attached by their convex margins to the wall of the vessel, and their free borders are directed forward in the lumen. The free and attached margins are strengthened by tendinous fibres "

The corresponding valves in *Varanus monitor* (Fig 10) bear an unmistakable resemblance to the figures given by Bhatia for *Uromastix hardwickii* I might, however supplement his description of them on the basis of a study of horizontal, longitudinal, as well as transverse sections.

The valves of the pulmonary trunk are the most posteriorly situated, those of the left systemic lie some distance anterior to them, and the ones pertaining to the right are the anteriormost. Posteriorly, the valves form a pair of more or less vertical septa (Fig 11), attached by their posterior

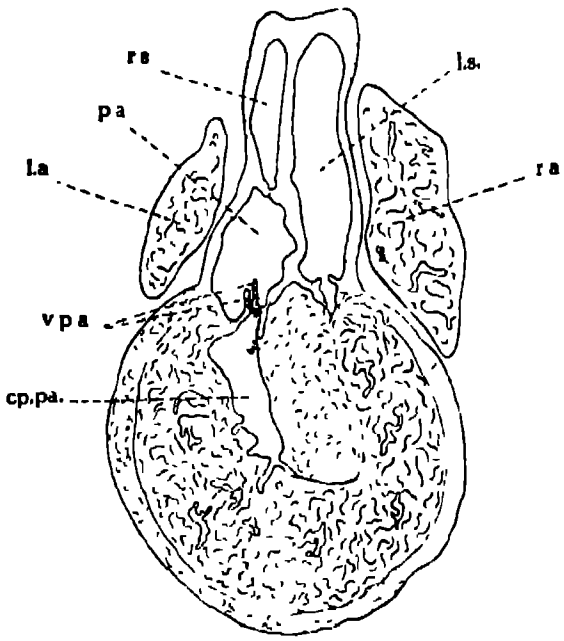


Fig 11. Horizontal longitudinal section through the heart of *Varanus monitor* showing the origin of the pulmonary and left systemic trunks with their valves (× 8)

v p a, valves of the pulmonary trunk, Other abbreviations as in previous figures.

convex borders in such a way as to separate, within the base of the aortic trunk, two lateral chambers from a central one (Fig. 10). Each lateral chamber forms a *cul-de-sac* opening only forwards into the lumen of the trunk. The central chamber is a laterally compressed, almost vertical space, communicating dorsally and dorso-posteriorly with the ventricular cavity.

All these chambers lead forwards into the root of the aortic trunk. The valves, as pointed out by Bhatia in *Uromastix hardwickii* (1929), are semilunar in appearance ; their straight borders are directed anteriorly and are free, while their convex borders are attached to the ventricular wall at the place of origin of the aortic trunk.

The arterial trunks, as is usual in reptiles, shift in their relative positions to each other as they run forwards on the ventral aspect of the heart and emerge from the pericardial cavity The pulmonary trunk changes from its ventro-sinistral position to a dorsal one, the left systemic trunk twists over from its origin on the right ventral side to the left side, and the right systemic descends from its dorso-lateral position on the right side to the definitely lateral position on the right.

9. *Summary*

The author has studied the minute structure of the heart in *Varanus monitor* (Linn) by means of transverse and longitudinal sections supplemented with dissections under a microscope and has thereby added a considerable number of new facts to our knowledge of this organ. The more important facts are as follows:

(1) The manner of opening of the sinus venosus into the right auricle has been minutely described in *Varanus monitor*. A suspending ligament (*nom. nov.*)[4] and a sinu-atrial channel (*nom. nov.*) are features not hitherto described in any lizard.

(2) The incorporation of the posterior parts of the two atria into the lumen of the ventricle as *atrium dextrum intraventriculare* (*nom. nov.*) and *atrium sinistrum intraventriculare* (*nom. nov.*) is an important feature, described for the first time in any vertebrate heart.

(3) The *atrium sinistrum intraventriculare* in *Varanus monitor* extends far backwards in the lumen of the ventricle, and the definitive left *atrioventricular aperture* (distinguished by the presence and position of its valves) opens much posteriorly into the *cavum dorsale* (*nom. nov.*) on the left side. There is no *cavum sinistrum* (*nom. nov.*) in the ventricle.

(4) The four regions of the ventricle, recognised in *Hemidactylus flaviviridis* by Mahendra (1942), are present in *Varanus monitor* also, but it is found that they are subject to a great deal of shifting in their relative position.

[4] The new names introduced by the author are distinguished by the abbreviation, *nom nov.* (= *nomen novum*), inserted in brackets after them.

(5) The apical region of the ventricle is divided into a dorsal space, the *cavum apicis dorsale* (*nom. nov.*) and a ventral space, the *cavum apicis ventrale* (*nom. nov.*) by a *horizontal septum.*

(6) Careful study of transverse sections leaves no doubt that the muscular ridge (' Muskelleiste ' of German Workers) in *Varanus monitor* as well as in other reptiles cannot be regarded as a dorso-ventrally disposed structure, as was believed by previous authors, but extends horizontally, arising as a conspicuous ledge-shaped structure from the lower part of the left wall of the ventricle and having its free border towards its right. The identification of this ridge as the *septum ventriculorum* in its entirety (Goodrich, 1916 and O'Donoghue, 1918) has been criticised.

(7) While, retaining the term ' *cavum pulmonale* ', used by previous workers, it has been found necessary to give up the terms ' *cavum arteriosum* ' and ' *cavum venosum* ' as they refer to the kinds of blood, though not quite correctly, they are assumed to deal with and affect, *a priori*, the interpretation of the *modus operandi* of the ventricle. When the space dorsal to the muscular ridge is single and undivided, it is designated by the author as the *cavum dorsale.* In certain cases, however, it may be completely or incompletely divided into a right and a left part (*cavum dextrum, nom. nov.* and *cavum sinistrum, nom. nov.*)

(8) A detailed description of the exact position of the atrio-ventricular apertures and of the disposition of their valves is given.

(9) The *pulmonary trunk* is the posteriormost to be delimited by an enclosure of the *cavum pulmonale* A little anterior to this, the left *systemic trunk* gets enclosed between the free border of the muscular ridge and the opposite wall of the ventricle. The base of the right systemic lies dorsal to that of the left and is formed by an anterior extension of the *cavum dorsale.*

10 Acknowledgments

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SOME HELMINTH PARASITES OF POULTRY

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Some of the parasites described in this communication were collected at Mukteswar and Izatnagar, some were received from various other localities in India, while two were sent from Singapore.

1. Nematoda

Acuaria (*Cheilospirura*) *hamulosa*, Diesing (1851) Railliet, Henry and Sisoff, 1912

Synonym A. (*C*) *pavonis* Ortlepp, 1925

This species is cosmopolitan in its distribution, occurring in the gizzard of the fowl and turkey, beneath the horny lining or in burrows in the muscular wall, on the surface of which it occasionally produces small nodules Baylis (1939) has summarised the descriptions of this species given by Tubangui (1926), Cram (1927–1931) and Li (1934). A comparison of the resume of the description of this species as given by Baylis with the description of *Acuaria* (*Cheilospirura*) *pavonis* Ortlepp, 1925, reveals the identity of these two species. When Ortlepp (1925) created the species *A. pavonis* for the specimens obtained from a Burmese Pea-fowl, the species *A. hamulosa* was not properly described and it would appear that Ortlepp was fully justified in propounding his new species Subsequent to his publication, a fuller account of *A. hamulosa* has become available and it has been observed that this species is very variable. Ortlepp separated his species from *A. hamulosa* on account of the different lengths of the cordons and the different arrangement of the post-anal papillæ Both these points of difference, however, are subject to variation, as will be apparent from the summary of the descriptions of this species as given by Baylis and from observations recorded below. For this reason it is considered necessary to regard *A. pavonis* as a synonym of *A. hamulosa*.

A few observations based on the examination of material obtained from a fowl in Baroda are recorded here.

The males measure 9·25–4 mm. in length and their breadth at the level of the posterior end of the œsophagus is 0·28–0·29 mm. In one specimen the pairs of cordons ended in the region of the anterior half of the left spicule, but at different levels. In another, one pair terminated slightly in front of the left spicule, while the other did so slightly behind the level of the anterior end of the same spicule The pharynx is 0·18–0·192 mm., the anterior portion of the œsophagus 0·7–0·74 mm. and the posterior part of the œsophagus 2·03–2 38 mm long, respectively The nerve ring is situated at 0 272–0·292 mm. from the anterior end. The anus lies at 0·385–0·432 mm. from the posterior end The right spicule measures 0 23 mm. in length and the left 1·435–1 45 mm. There are four pairs of pre-anal and six pairs of post-anal papillæ. The arrangement of the post-anal papillæ was not quite typical with the records of Baylis but it was found to differ from specimen to specimen and even in the same specimen the arrangement differ on the two sides. Thus in one specimen examined the first three pairs were exactly as described by Baylis but the arrangement of the last three papillæ was different. The last pair of papillæ were situated at 0·039 mm from of the tip of the tail, the fifth pair of papillæ were situated at 0·068 mm. in front of the last pair and the fourth pair were situated at 0·066 mm. in front of the fifth pair. In another specimen the arrangement on one side was as described above but on the other side the last two papillæ were very close together and the others were situated at different levels.

The female is 13·15 mm. long and 0·39 mm. broad in the region of the posterior end of the œsophagus The cordons terminate about 1–3 mm. behind the vulva and at slightly different levels. The pharynx is 0·22 mm., the anterior part of the œsophagus 0·67 mm. and the posterior part 2·6 mm. long respectively. The nerve ring is situated at 0·346 mm. behind the anterior end. The vulva is situated at 5·15 mm. and the anus at 0·32 mm. from the posterior end. The eggs measure 0·0315–0·0325 × 0·018–0·021 mm.

Acuaria (*Dispharynx*) *spiralis* (Molin, 1815) Railliet, Henry and Sisoff, 1912

Although this species has a very wide distribution, it has been recorded only once in this country by Maplestone (1932) from a bronze-winged jacana, *Metopidius indicus*. A few specimens were received from the nodules in the proventriculus of a fowl in Hyderabad (Deccan), which on examination were found to belong to this species. This is the first record of the occurrence of this species in a domestic fowl in India. Observations

made on specimens at our disposal conform to the consolidated account of this species given by Baylis (1939).

Tetrameres mohtedai n.sp.

Eight females and ten males of *Tetrameres*, obtained from the proventriculus of a fowl in Hyderabad (Deccan), were sent by Mr. Nurul Mohteda, G M.V.C., Asst. Veterinary Investigation Officer, Hyderabad, which on close examination proved to be a species new to science

The male measures 4 27–5·8 mm. in length. The cuticle is transversely striated. A very small anterior portion of the body is devoid of spines. Behind this, the whole body is armed with two almost lateral rows of spines. In addition to these two rows there are two submedian longitudinal rows of spines extending to slightly behind the middle of the posterior portion of the œsophagus. The spines are more closely situated in the anterior and posterior parts of the body than in the middle part. The lips are thick and bi-lobed and bear two papillæ The dorsal and the ventral cephalic shields are present The lateral alæ arise at the base of the lip and extend behind for a very short distance " Cordons " arise from small prominences on each side of the head, from which they pursue a slightly wavy posterior course. They fuse with the body-wall slightly behind the middle of the posterior portion of the œsophagus. The cervical papillæ are situated at 0·268–0 290 mm. from the anterior region of the body. The buccal capsule measures 20–27 mm. in length and 8–23 mm. in width. The anterior portion of the œsophagus is 0 231–0·450 mm. and the posterior portion 0·73–1·33 mm. long. The nerve ring is situated at 0·15 mm. from the anterior end of the body.

The tail is straight and is 0·290–0·344 mm. long. There are no caudal alæ or papillæ, but subventrally on each side there is a row of five spines. In front of the cloacal aperture there is on each side a single row of closely situated spines in the mid-ventral line. The longer spicule is 0·397–0·430 mm. long and has a characteristic shape as shown in the figure. The smaller spicule measures 0·142–0·160 in length and is curved. Both the spicules terminate in rounded extremities.

The female is 3·24–5·64 mm. long and 1·94–3·22 mm. wide. The body is globular, blood-red in colour and has four longitudinal furrows corresponding to lateral and median lines. The anterior extremity protrudes from the globular body for a length of 0·9–1·73 mm. and the protruding posterior part of the body is 0·51–0·88 mm. long. The buccal capsule is 0·017–0·018 mm. long and 0·014 mm. wide. The anterior

part of the œsophagus is 0·267–0·34 mm. and the posterior part 1 15–1·16 mm. long. The cervical papillæ are situated at 0·188–0·191 mm. and the nerve ring at 0·186 mm. from the anterior extremity of the body. The intestine is saccular and is filled with black detritus. The anus is situated at 0·18–0·335 mm. and the vulva at 0·32–0·6 mm. from the posterior extremity

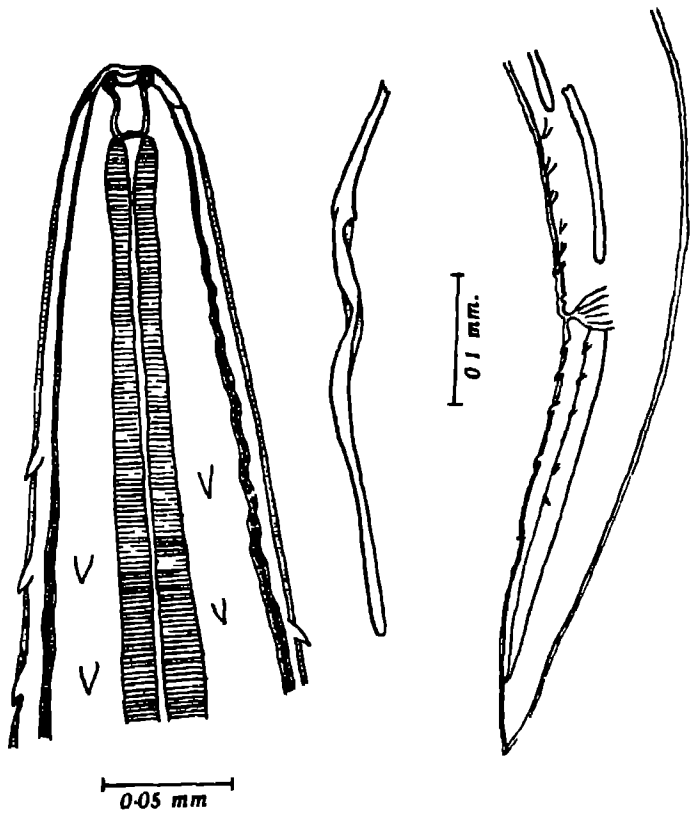

FIG 1 FIG 2 FIG. 3

Fig. 1 *Tetrameres mohtedai*, Anterior end

Fig 2 *Tetrameres mohtedai*, Spicule.

Fig 3. *Tetrameres mohtedai*, Lateral view of the posterior end of male

of the body. The copulatory receptaculum is 0·11–0·15 mm. long. The ovaries and the uteri are very long and their coils fill up most of the body cavity. The eggs measure 0·043–0·055 × 0·029–0·032 mm.

So far only three species of *Tetrameres* have been recorded from the fowl, *viz.*, *T. fissispina*, *T. confusa* and *T. americana*. The parasites at our disposal presented points of resemblance to the three species named but were not fully identical as may be seen from the table.

TABLE I

	T mohtedai	*T fissispina*	*T confusa*	*T. americana*
Male—				
Length	4·27-5 8 mm	3-6 mm	4 5 mm	5-5 5 mm.
Vestibule	20–27-μ × 8-23 μ	8 × 3 μ	24–80 μ	27 × 4·5 μ
Pharynx	0 235-0 450 mm		0 250 mm	0 365 mm
Oesophagus	0·730-1 330 mm	0 78 mm	0 740-0 990 mm.	0 996-1 360 mm.
Cervical papillæ	0 268-0 290 mm	0 150 mm		0·183-0 189 mm
Large spicule	0 397-0 430 mm	0 280-0 490 mm	0 291 mm	0 290-0·312 mm.
Small spicule	0 142-0 160 mm	0 082-0 150 mm	0 068 mm	0 100 mm.
Cloaca	0 290-0 344 mm	0·130-0·250 mm	0 070 mm	0 232-0 290 mm.
Female -				
Length	3 240-5 64- mm	1 67-6 mm	3-5 mm	3 5-4·5 mm
Vestibule	17-18 × 14 μ	21 × 10 μ	20 × 14 μ	35-38 × 10 μ
Pharynx	0 267-0 340 mm	0·230 mm	0 400 mm	0 300-1 315 mm
Oesophagus	1 150-1 160 mm	1 230 mm	2-2 4 mm	1 4 mm
Anus	0 180-0 335 mm.	0 071 mm	0 250 mm	0·332 mm
Vulva	0 320-0·600 mm	0 310 mm	Near anus	0·631-0 664 mm.
Copulatory receptaculum	0 110-0 150 mm	0·400 mm		0·274 mm
Eggs	45–55 × 29-32 μ	45–56 × 26-30 μ	33 × 24 μ	42-50 × 25 μ

It will be apparent from this table that the species in question differs from *T. fissispina* in the dimensions of its buccal capsule, the positions of the cervical papillæ and the cloaca of the male and the position of the anus and the length of the copulatory receptaculum of the female. From *T. confusa* it differs in respect of the dimensions of the buccal capsule, the length of the two spicules, in the position of the cloaca of the male, in the length of of the œsophagus of the female, and in the dimensions of the eggs. It differs from *T. americana* in the lengths of the spicules, in the position of the cervical papillæ of the male, in the dimensions of the buccal capsule and in the lengths of the œsophagus and the copulatory receptaculum of the female. For these reasons it is considered necessary to regard the material described here as belonging to a new species for which the name *Tetrameres mohtedai* is proposed.

Capillaria columbæ (Rud., 1819) Travassos, 1915

This parasite occurs in the small intestine of the domestic pigeon fowl and turkey in many parts of the world. In India it has been recorded so far only from the intestine of a pigeon by Baylis and Daubney (1922). Recently, an opportunity was afforded to examine these worms obtained from a pigeon in the Nizam's Dominions and fowls in Bombay Presidency and the United Provinces. The following observations are recorded on the material:—

The male measures 9·2 mm. in length and the œsophageal portion is 4·75 mm. long. The spicule measures 1·3–1·39 mm. in length. The

spicular sheath is transversely wrinkled and posteriorly appears to be distinctly striated. In the fully extruded condition the sheath appears to be very long, and measuring 1·47–3 49 mm. in length.

The female measures 12–7 mm. in length and the œsophagus 5 mm. The vulva is situated at 0·084 mm. behind the junction of the œsophagus and the intestine. The eggs, including the polar plugs, measure 0·048–0·055 × 0·024–0·029 mm.

Bhalfilaria badamli n.g., n.sp.

One female recovered from the heart of a Black Minorca cock at Patanchera, sent by the Director of Veterinary Services, Nizam's Dominions, was available for study.

The worm is filiform with blunt ends. It measures 34·8 mm. in length and its breadth in the region of the head is 0·105 mm., in the mid-body 0·501 mm. and in the region of the anus 0·173 mm. The cuticle of the worm has conspicuous longitudinal striations at varying distances apart. The mouth is simple and has no lip-like structures. The anterior extremity has two lateral and four subventral papillæ. The œsophagus is distinctly divided into a narrower muscular portion, measuring 0·435 mm in length, and a somewhat wider glandular portion which is 1·93 mm. long. The nerve ring is situated at 0·26 mm from the anterior end. The excretory pore is situated behind the middle of the glandular portion of the œsophagus at a distance of 1·5 mm. behind the anterior extremity. The tail gradually tapers to a blunt point and the anus is situated at a distance of 0·147 mm. from the posterior extremity. On each side of the anus there are two lateral papillæ. At a distance of 0·113 mm. in front of the anus there is a pair of large papillæ. By comparison, the rectum is much narrower.

The ovaries are comparatively much smaller and both of them lie in the posterior part of the worm. The anterior ovary is straight, but the posterior is reflexed. The posterior ovary begins at 1 mm. and the anterior at 4·3 mm. from the hinder end of the worm. The oviducts are also small. The uteri are wide and run almost parallel to one another for the greater part of their course but for a short distance anteriorly they are coiled spirally around each other. The vulva and vagina were not seen in spite of prolonged observations. Microfilaræ which are more or less spindle-shaped and measure ·020–·024 × ·0022–·004 mm.

The worms recorded so far from the heart of birds are *Splendidofilaria pavlowskyi* Skrjabin, 1923, *Chandlerella bosei* (Chandler, 1924) Yorke and Maplestone, 1926, *Macdonaldius carinii* Vaz and Pereira, 1935 and *Paronchocerca ciconiarum* Peters, 1936. *Cardiofilaria pavlowskyi* Strom, 1937,

has been recorded from the pericardial cavity of *Oriolus oriolus* Kundoo. Of these five genera of Filariidæ, the specimen described here agrees with *Macdonaldius* since in this genus also the position of the vulva is not discernible: the other four genera having a distinct vulva. It, however, differs from *Macdonaldius* in many respects, and for this reason the present form may be tentatively assigned to a new genus for which the name *Bhalfilaria*

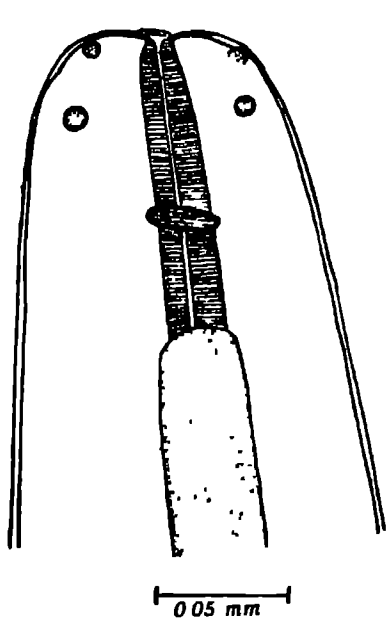

FIG. 4

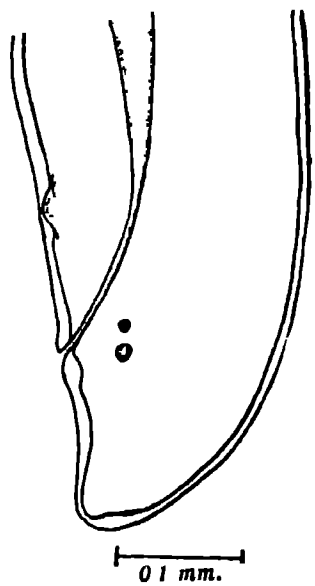

FIG. 5

Fig 4 *Bhalfilaria badamii*, Anterior end

Fig 5 *Bhalfilaria badamii*, lateral view of the posterior end of female.

is proposed. (The species is named *Bhalfilaria badamii* in honour of Rao Bahadur B. K. Badami, G.B.V.C, Director of Veterinary Services, Hyderabad-Deccan.) The task of assigning this genus to one of the subfamilies of Filariidæ may await the discovery of a male worm and examination of some more forms.

Heterakis beramporia Lane, 1914

Synonym—*Heterakis putaustralis* of Maplestone, 1932

This species was originally recorded from the cæca of a fowl in India by Lane (1914). Subsequently; it was recorded from China, Burma and the Philippines. Baylis (1936) pointed out that the species *H. putaustralis* of

Maplestone, 1932 is probably a synonym of *H. beramporia*. A few years ago some specimens of this species were received from Singapore. It would therefore appear that this species has a wide distribution in the Orient. In a few respects the Singapore material differs from that described so far. The right spicule is 0·373–0·378 mm. long and the left spicule is 0·276–0·32 mm. The genital sucker measures 0·062 mm. in diameter In female worm measuring 5·91 mm in length, the vulva is situated at 3·38 mm. from the anterior end.

2. Cestoda

R. rangoonica Subramanian, 1929

This species was originally described by Subramanian (1929) from a fowl in Rangoon. Subramanian laid stress of the length on the cirrus sac which in the Rangoon material was 0·096 mm long and amongst other things this criterion was used by him to distinguish it from *R. retractilis*, in which the organ is stated to be 0·12 mm long. Some material of this species was collected from a fowl at Mukteswar which agreed in all respects with the description given by Subramanian, but the cirrus sac in these specimens was 0·13 mm long The length of the cirrus sac appears therefore to be variable in this species.

R. (*Railletina*) *grobbeni* Böhm, 1925

Although Lopez-Neyra (1931) regarded this as a distinctly valid species, Fuhrmann (1932) and Neveu-Lemaire (1936) regard it as a synonym of *R. echinobothridia*. Material of this species was collected from fowls at Izatnagar. The worms are 200–30 mm. long There are about 200 rostellar hooks, the large hooks measuring 0·013 mm. and the smaller ones 0·011 mm. in length. The acetabular hooks are deciduous. In one specimen 8–9 rows of acetabular hooks were noticed. The genital pores are unilateral and open in the posterior half of the proglottis margin. Previous authors have failed to give measurements of the cirrus sac but in our material it measured 0·172–0·23 × 0 09 mm. The testes were found to be 25–34 in number. Each egg capsule contains only 3–6 eggs.

From the observations given above it will be apparent that *R. grobbeni* is very much allied to *R. echinobothridia*. The only point of outstanding difference is the number of eggs in the egg capsules, which in the case of *R. echinobothrida* are 6–12 in number, while we could detect only 3–6 eggs in each egg capsule of *R. grobbeni*. It is therefore doubted whether this difference, coupled with the slightly larger cirrus sac, is sufficient for its separation from *R. echinobothrida*.

R. tetragona (Molin, 1858)

This was the commonest species of cestode encountered in fowls at Izatnagar and Mukteswar. On examination of a large number of specimens it was felt that the previous account of this species could be supplemented in respect of the acetabular hooks, the number of testes and in respect of the length of the cirrus sac. The specimen at our disposal measured 240–50 mm. in length, the rostellar hooks are 100, each measuring 0·006–0·007 mm. The acetabular hooks were quite typical in some cases, but in others a few or more were found missing. In one case, however, all four acetabula were found completely devoid of spines. The genital pores were unilateral and were situated in the anterior half of the proglottis margin. The testes range from 25–38, but in most cases they were 30–35. The cirrus sac measures 0·08–0 14 mm There are 60–120 egg capsules in each segment and each capsule contains 6–12 eggs The eggs measure 0·03–0 06 mm. in diameter.

Raillietina (*Raillietina*) *echinobothrida* (Megnin, 1881)

Although this species is cosmopolitan in distribution, it has not so far been recorded from Singapore. A few years ago some scolices of tapeworms of a fowl in Singapore was received which on examination proved to be those of *R. echinobothrida.*

Cotugnia digonopora (Pasquale, 1890) Diamare, 1893

This is a common parasite of fowls in India. Most authors state that the rostellum in this species bears an enormous number of minute hooks, arranged in a double row, while Southwell (1930) maintains that the hooks on the rostellum are disposed in a single row. Numerous specimens of this species were examinad with a view to obtain exact information on this point. All the worms examined, however, had two rows of hooks on the rostellum.

Amæbotænia sphenoides (Raillet, 1892) Cohn, 1899

In this country, this species has so far been recorded from Bengal. Recently, we examined worms of this species from fowls in Assam and the United Provinces, and this material conformed to the description of previous authors.

Hymenolepis cantaniana (Polonio, 1860)

Although this species has been recorded from the Far East, Europe and America, it has not been so far known to occur in India. Material of this species was collected from a large number of fowls dissected at Izatnagar. Nearly 20% of the fowls were infested and the infestation was heavy and confined to the duodenum. In most cases it was extremely difficult to extricate the scolex from the mucous membrane, but, if the duodenum is

slit open and left in luke-warm water for some time the scolices come off more easily. The worms at our disposal measured up to 27·9 mm. in length. The rostellum was unarmed and measure 0·06–0·087 mm. in diameter. The cirrus sac is 0·073–0·106 mm. long. In the previous description of this species, it has been stated that the cirrus sac extends up to the middle of the proglottis, but in nearly half the cases it extends only upto one-third of the width of the proglottis, while in others it extends to the middle. The eggs measure 0·055–0·0575 mm. in diameter and the hooks of the onchosphere are 0·013 mm. long.

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VINE CHILLY—A COMPARATIVE MORPHOLOGICAL AND CYTOLOGICAL STUDY

By L. S. Dorasami and D. M. Gopinath

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(Communicated by Prof L S S Kumar, F A Sc)

In a recent article an unusual vine chilly plant was reported by Narasimhan[1] on the basis of the material collected near Goribidnur, Mysore State. A new variety, *viz.*, *Capsicum frutescens* var *scandens* has been proposed for its accommodation. In designating the new variety, a comparative account of the anatomy of the leaves, stem and roots, as also the sizes of the stomata and pollen of the vine chilly plants and the local varieties grown near Goribidnur were taken into account by Narasimhan. Since no appreciable differences were manifest, he concluded that the scandent habit of the vine chilly plants must have arisen by way of simple gene mutation. As there were no cytological studies made to confirm this interesting feature, the writers undertook the study of the chromosome numbers and their behaviour, an account of which is presented here A detailed morphological study of the male and female gametophytes of the vine chilly plants and of the normal plants grown locally were made with a view to institute further comparisons.

The material for study was collected from the same vine chilly plants which formed the material for study by Narasimhan From the same locality, the material for the normal chilly plants, which served as control, were collected. Material for microscopic study was fixed in "Craf" recommended by Randolph with pretreatment in Carnoy's fluid for ten minutes. Sections were stained with Newton's iodine gentian violet method. Chromosome studies were also made in aceto-carmine smears.

As regards the structure and development of the microsporangium, no differences have been observed between the vine chilly plants and the local varieties which were taken as controls. In fact the microsporangium in both the cases is quadrilocular, the development of the pollen from the initial stages proceeding according to the normal type. The distinction between the wall layers and the endothecium is not apparent. The tapetal cells in both the plants are enlarged even to the extent of being slightly hypertrophied, the cells showing two conspicuous nuclei and deeply staining

cell contents. However, in the vine chilly plants the tapetal cells are slightly globoid and abutting on the sides, as against the isodiametric tepetal cells of the control plants (Figs. 1 and 4).

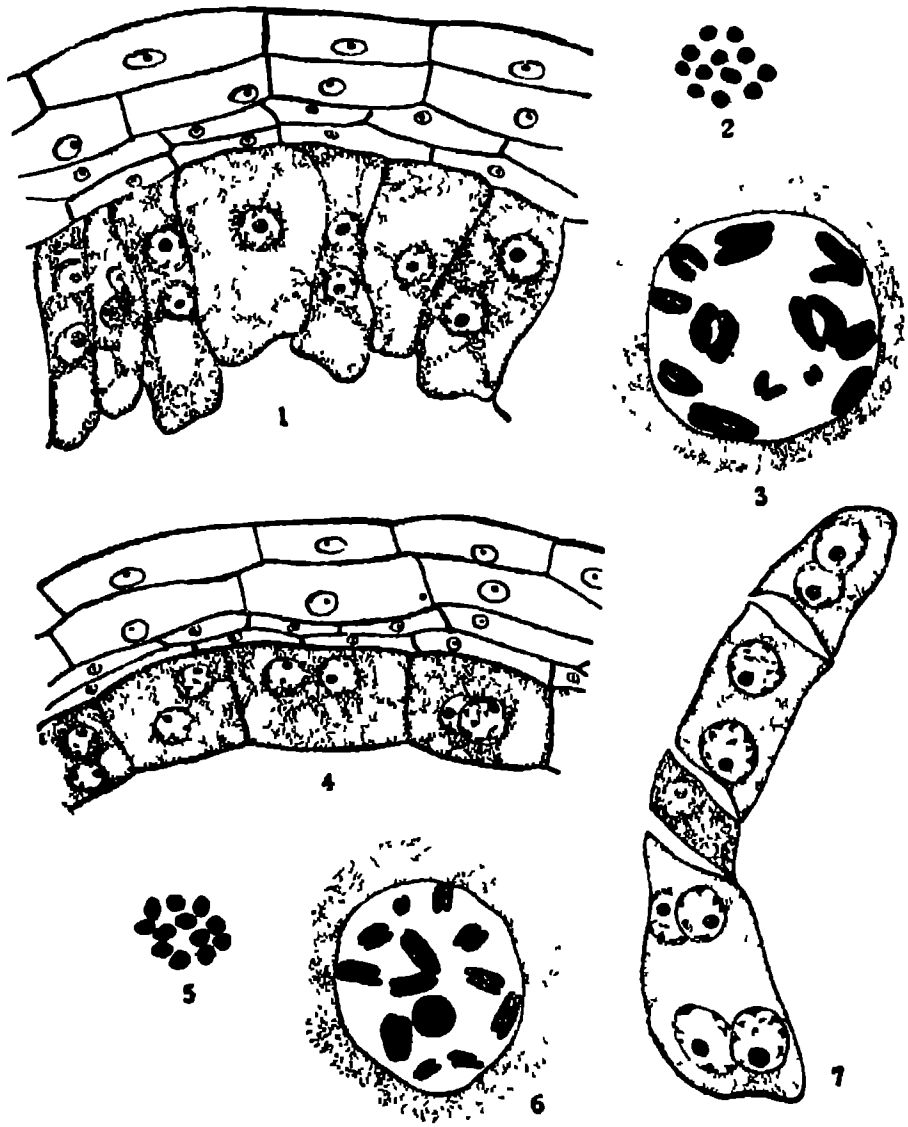

Fig 1.—Tapetal cells of the vine chilly anthers × 528. Figs 2 and 3. Metaphase plate and diakinesis in the pollen mother cells of the vine chilly plants × 1900. Fig. 4. Tapetal cells of the control plants. × 528 Figs. 5 and 6 Stages in the meiotic division of the pollen-mother cells in the control. × 1900. Fig. 7. Showing the simultaneous development of all the megaspores in the vine chilly plants. × 900.

The meiosis is normal in both the plants under investigation and neither lagging of the chromosomes nor other abnormalities associated with the heterozygous forms have been observed. The haploid number of chromosomes in both the plants is twelve (Figs 2, 3, 5 and 6). This is in conformity with the number given by Dixit for *Capsicum annuum*.[1] The length and the shape of the chromosomes as determined and seen at various phases of the nuclear divisions are identical in both the cases

A comparative study of the megasporogenesis of the vine chilly and control plants indicated a similar type of development. The type of embryo-sac conforms to the normal 8-nucleate type. The ovules are anatropous with a single integument. It is not the object of the writers to give any detailed account of the gametogenesis but only a comparative account as regards the development of the gametophytes in the two plants. After the formation of the linear row of tetrads of megaspores only the chalazal megaspore is functional. As against this, in the vine chilly plants simultaneous development of all the megaspores up to the 2-nucleate-embryo-sac stage has been noticed occasionally (Fig 7). But such differences in the development of the megaspores might not have any significance in differentiating varieties. In both the plants the antipodals in the mature embryo-sac are cellular and synergid-like. Fertilization is of the normal porogamous type.

From the above studies it becomes manifest that the vine chilly plants and the local varieties grown closely resemble each other as regards gametogenesis, size, shape and number of chromosomes. The minor differences noticed such as, shape of the tapetal cells, occasional development of all the megaspores in the vine chilly plants are not of such value in distinguishing the varieties. The homozygous nature of the vine chilly plants, and their close resemblance with the local varieties of chilly plants as regards their morphological and anatomical features as pointed out by Narasimhan, leads to the conclusion that the vine chilly plants must have arisen only by simple gene mutation.

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ASPERGILLOSIS IN FOWLS

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Received March, 18, 1944
(Communicated by Rao Bahadur Dr D V Bal, F A Sc)

For the first time in this Province a serious and fatal outbreak of Aspergillosis in fowls has been encountered with during 1943. The disease was first brought to the notice of the author by Mr G. D Pani, Poultry Investigation Officer, and a systematic study of the disease has been taken up.

A. C Mayer first observed the disease in 1815 in the bronchi and air-sacs of jay. Henry Grev has also mentioned that the disease is common in Great Britain. Thom and Church report that *Aspergillus fumigatus* caused death in birds in zoological gardens in different countries. Since then several other writers have mentioned cases of affection in different wild species of birds and domestic poultry However, as far as known to the author this disease has not so far been reported from anywhere else in this country

The outbreak of Aspergillosis was first detected in some fowls at Dhamtari during the end of May 1943 Severe and fatal outbreaks of the disease were later on reported from Government Poultry Farm, Telankheri, Nagpur, in chickens of 4 to 6 months old and practically all the young birds died during June, July and August. The disease was again reported in an epidemic form from Raipur, Khandwa, Chhindwara, Seoni and several other places in the Province. It was also observed that Rhode Island Red were more susceptable to the disease than Deshi or Black Minorca; White Leghorns being the most resistant of all the breeds. Mortality in chickens was very much higher than in adults.

During the course of investigation it was observed that in severe cases of infection the birds die overnight though apparently healthy the previous day. In less acute case the sick birds became dull and droopy with rise of body temperature, preferred darkness to light, lost appetite, and developed paralysis of the legs and wings. The birds exhibit frequent sneezing and coughing with rattle in the throat and gasp for breath. There is slimy discharge from nose and beak; the eyelids swell and have cheesy deposits. In later stages of the disease the bird develops diarrhœa and the feathers

at the vent are soiled Such birds waste in condition and generally die within a week. In other less severe cases of infection the birds develop the above symptoms gradually and die within three to four weeks due to exhaustion.

The other common features which were observed in the diseased birds were paleness of body flesh, greyish necrotic growths and lumps of cheesy material in the lungs and air-sacs In some cases bluish-green necrotic patches were found in the lungs while in others blood clots were noted when cut open, though appeared normal externally. Trachea and bronchi may show hæmorrhage along with cheesy deposits The lumen of infected œsophagus was studded with greyish nodules resembling vitamin 'A' deffi-ciency lesions The proventriculus may show hæmorrhage patches along with bluish-green growth at the junction of the proventriculus to the extension of the crop The infected intestines were thickened. It was common to find both the cæcal pouches filled with cheesy puss with hæmor-rhage of mucus membrane. Cheesy deposits were prominent all over the abdominal cavity when peritonium was infected. Kidneys, ovaries and testes when infected were considerably enlarged and showed greyish or bluish-green deposits. Congestion of heart and hæmorrhages in bone-marrow and brain was of common occurrence.

Under sterile conditions lesions from all the infected organs were cultured on broth, rice-mesh agar and Sabouraud's media and practically in every case pure cultures of *Aspergillus sp.* were obtained. In several cases heart and cutaneous blood, when cultured on starch agar, gave a luxuriant growth of *Aspergillus sp.* within ten to twenty days. The fungus when cultured on slightly acidic medium is bluish-green in colour but it forms brown colonies on alkaline medium.

To prove the pathogenicity pure cultures of the isolated *Aspergillus sp.* were obtained and the disease was artificially transmitted to the healthy fowls in the following three ways· (1) spores were mixed with grain and the birds were fed on them; (2) spores were insuflated into the nostrils of healthy birds; (3) sub-cutaneous injections were carried out on the birds by a spore suspension in sterile distilled water. In the first two cases the experimental birds died on the 26th and 28th day respectively while by the third method death took place on the 11th day. Typical Aspergillosis symptoms were observed in all the experimental birds and on reisolation the same species of *Aspergillus* was obtained from the lesions.

A general study of the pathogen was taken up. The fungal colonies on rice-mesh agar are velvety to felted floccose, spreading, yellowish-green

to dark green to almost dark-brown in age. Reverse and substratum are colourless to yellow and occasionally reddish-brown to red in old cultures. The vegetative hyphæ are septate, hyaline and measure 2·8-3 2 μ in breadth (varying from 2·4-3 5 μ) Conidiophores are short, septate, densely crowded, light green in colour specially above, arise either from submerged or aerial hyphæ and enlarge towards the top into a flask-shaped vesicle. They measure 4 9-6 3 μ in breadth (varying from 4 2-6 6 μ) at their source, 9·1-9·8 μ (varying from 7-10 5 μ) in the middle and 11·2-12 6 μ (varying from 9·1-13 3 μ) at the base of the vesicle The length of mature conidiophore is 329-350 μ (varying from 215 5-350 μ) The vesicle is smooth, green in colour, usually fertile only on the upper half and measure 18·9 21·7 μ in diameter (varying from 18·9-27·3 μ). All over the round part of the vesicle closely packed green coloured sterigmata are arranged in one series, run approximately parallel to the axis of conidiophores, bear conidia in chains and measure 6·3-7 7 μ × 2 4-2 8 μ (varying from 5 6-8 4 μ × 2 1-2 8 μ). Single conidium is faint green in colour but in mass dark-green. They are smooth, spherical and measure 2 8-3 2 μ in diameter (varying from 2·4-3 5 μ). The older conidia have thick and roughened wall (Fig. 1).

This species of *Aspergillus* is very much similar to *Aspergillus fumigatus var. alpha.* Sion and Alexandresen in its cultural and pathogenic behavior. Dodge[1] and Thom and Church[2] have mentioned that this species of *Aspergillus* apparently cause severe epizootics in birds, less fatal in man, not reaching in epidemic proportions and pathogenic to laboratory animals. During the course of study it was observed that this species of *Aspergillus* is pathogenic to human beings, goats and rats as well though not to the same severity as in fowls Mr. G. D. Pani while conducting certain feeding experiments with this pathogen accidentally got infected on his left cheek where probably there was a small cut due to shaving An abscess developed that place with severe pain in back, feverishness, pain in the left cheek, at nausea and sleeplessness. The puss from his abscess was examined by the author and it was found full of spores which on culturing and examination proved to be that of *Aspergillus fumigatus.* Mr. Pani improved by tincture-iodine injections but subsequently had to proceed to the Tropical School of Medicine, Calcutta, for proper treatment.

The fungus thrives best during warm humid climates of this Province. Long periods of atomospheric humidity causes the fungus to thrive well and mostly during such periods epidemic of Aspergillosis is exhibited in fowls. Samples of poultry feed, *viz.*, grain and mash, from Government Poultry Farm, Telankheri, were examined for fungoid growth On culturing

and examination the specimens were found infected with *Aspergillus fumigatus*. The spores of the pathogen are abundantly present in dry grass, damp soil, grain and mash during wet weather and gain access to the body by inhalation.

When heart and cutaneous blood from diseased fowls gave positive results for *Aspergillus fumigatus*, it was thought desirable to examine and culture the different parts of the eggs from infected pens Eggs from infected and healthy pens were obtained from the Government Poultry Farm, Telankheri, Nagpur, and hundreds of cultures were taken from the washings of the eggs, shells, shell membrane, albumin, chalaza and yolk *Aspergillus fumigatus* was obtained in most of the cases from the eggs of the infected pens from shell membrane, albumin and chalaza. In spite of several attempts the pathogen could not be isolated from yolk and shell. The cultures were taken either on rice-mesh agar or broth medium. Spring has isolated from eggs *Aspergillus glaucoides* and *Aspergillus heterocephalus* of which the former has been proved to be indistinguishable from *Aspergillus fumigatus* Fres. The *Aspergillus sp.* isolated from the inner contents of the eggs was slightly different from the species isolated from the diseased fowls. The hyphæ of such isolates is thread like, hyaline and measure 3·5–6·3 μ in breadth; the spores are roughened in outer wall, mostly sphærical, pale yellow in centre and dark brown at the periphery and measure 3·5–6·3 μ in diameter. Repeated sub-culturing however has later shown that it is indistinguishable from the species isolated from the lesions of diseased fowls. The difference exhibited at the early stage of isolation is most probably due to the different nature of the host. Transmission of the disease to healthy birds by the egg isolate could not be tried so far.

Sixteen fertile eggs from infected pen were kept for incubation but none of them hatched out. In a few cases dead embryos were seen on breaking the egg shells while in others opaque, non-motile, dense spot in the enlarged air-chamber were observed which on culturing gave colonies of *Aspergillus sp.* These experiments have shown that the disease is transmissible to the eggs when laid by sick birds. Further work on the disease is still in progress.

I am grateful to Mr. H. B. Shahi, Director of Veterinary Services, Government, C. P. and Berar, for according necessary facilities in this work. My sincerest thanks are due to Mr. G. D. Pani for bringing the disease to my notice and for his valuable help during the investigation. I am also thankful to Mr. R. A. Narke and Mr. S. L. Zargar for the help rendered by them.

Fig. 1. *Aspergillus fumigatus* ×900

THE DEVELOPMENT OF THE FEMALE GAMETOPHYTE IN SOME MEMBERS OF THE EUPHORBIACEÆ

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(Communicated by Prof G P Majumdar, F A Sc)

ALTHOUGH in recent years a considerable amount of embryological work has been done in India and abroad, the family Euphorbiaceæ has not, however, received the amount of attention it deserves, considering the large number of species it includes Schnarf[12] has reviewed the relevant literature on the subject upto the year 1931. According to him the general mode of development of the female gemetophyte is of the normal type A few abnormal types have been recorded and classified under different heads such as Phyllanthoideæ, Crotonoideæ, etc Later work has been recently reviewed by Maheshwari.[8]

Not much work has been done in India on this family. Maheshwari and Chowdry[7] published a short note on the development of the embryo-sac of *Phyllanthus niruri* collected from Agra. The mature embryo-sac appeared to be 4-nucleate due to the degeneration of the antipodals. Later Maheshwari and Johri[9] published a short account of the development of the female gematophyte in *Acalypha indica* and reported the occurrence of a tetraporic 16-nucleate embryo-sac of the "Penæa form" under the Peperomia type. The organization of the mature embryo-sac shows a great variation Miss Bhalla[2,3] studied the development of the female gametophyte in *Euphorbia helioscopia* and *Euphorbia Royleana*. In the former the archesporial cell is hypodermal in origin and cuts off an upper parietal cell and a lower megaspore mother cell. The latter gets buried deeply into the nucellus by the activity of the parietal cell. In *Euphorbia Royleana* she noticed sterility due to the non-development of the embryo-sac beyond the archesporial stage. The archesporial cell, however, is found buried 2–3 cells deep in the nucellus.

Very recently Maheshwari[6] has studied the development of the embryo-sac of *Euphorbia heterophylla*. This had been first investigated by Modilewski[10] who reported it to be of the Normal-type. Sanchez[11]

re-investigated it and found the embryo-sac to be tetraporic and 8-nucleate (*Adoxa type*). Maheshwari, however, reports a Normal-type of development. Kajale and Rao[4] have very recently described the development of the female gametophyte in *Euphorbia hirta* and *Jatropha gossypifolia*. They found the presence of an obturator and a normal type of development of the embryo-sac in both the plants.

Material and Methods

The following plants furnished the material on which the investigation is based :—

1. *Putranjiva Roxburghii* Wall.
2. *Trewia nudiflora* Linn.
3. *Phyllanthus niruri* Linn., and
4. *Euphorbia thymifolia* Burm.

Of these the first two are dicecious and are road-side trees commonly growing in Calcutta, while *Phyllanthus niruri* and *Euphorbia thymifolia* are small monœcious herbs. The former commonly occurs in waste fields, while the latter is found generally on the walls of old buildings

Young ovaries and flower buds at different stages of development were fixed in the field generally between 8–11 A M., and between 1–3 P.M. In advanced stages, the ovaries of *Putranjiva Roxburghii* and *Trewia nudiflora* become greatly enlarged and hence to facilitate quick and easy penetration of the fixing fluids, the superficial and unnecessary tissues of the ovarian walls were trimmed off carefully without injuring the ovules

Allen's modified Bouin's and Nawaschin's fluids were used for fixation. Both gave fairly good results but Nawaschin's fluid appeared to give slightly better results. In the case of *Euphorbia thymifolia* difficulties were experienced in fixing, possibly due to the presence of excessive latex in its tissues. The materials after fixation were dehydrated and cleared in the usual way, and finally imbedded in paraffin.

Sections were cut from 6–16 microns thick, the thickness depending on the stages of development required for study. Heidenhain's Iron-Alum Hæmatoxylin was used for staining.

Observations

(*a*) *The Integuments*

The ovules are anatropous and bitegmic. In *Putranjiva Roxburghii* and *Trewia nudiflora* both the integuments in the mature ovule take part in the formation of the micropyle which is fairly long. In *Phyllanthus*

niruri and *Euphorbia thymifolia*, however, no true micropyle is formed In the former, at the mature embryo-sac stage, the nucellar beak remains protruded beyond the integuments (Text-fig 8), while in *Euphorbia thymifolia* the nucellus and the integuments lie almost at the same level (Text-Fig 2).

The thickness of the integuments varies in the different plants, depending on the size of the ovules, large ovules having thicker integuments. Thus in *Putranjiva Roxburghii* and *Trewia nudiflora* the integuments were 3 to 9 cell layered, while in *Phyllanthus niruri* and *Euphorbia thymifolia* they were 2 to 4 layered. In *Trewia nudiflora* the outer integument becomes so massive that at first sight the ovules appear to be covered by a single integument.

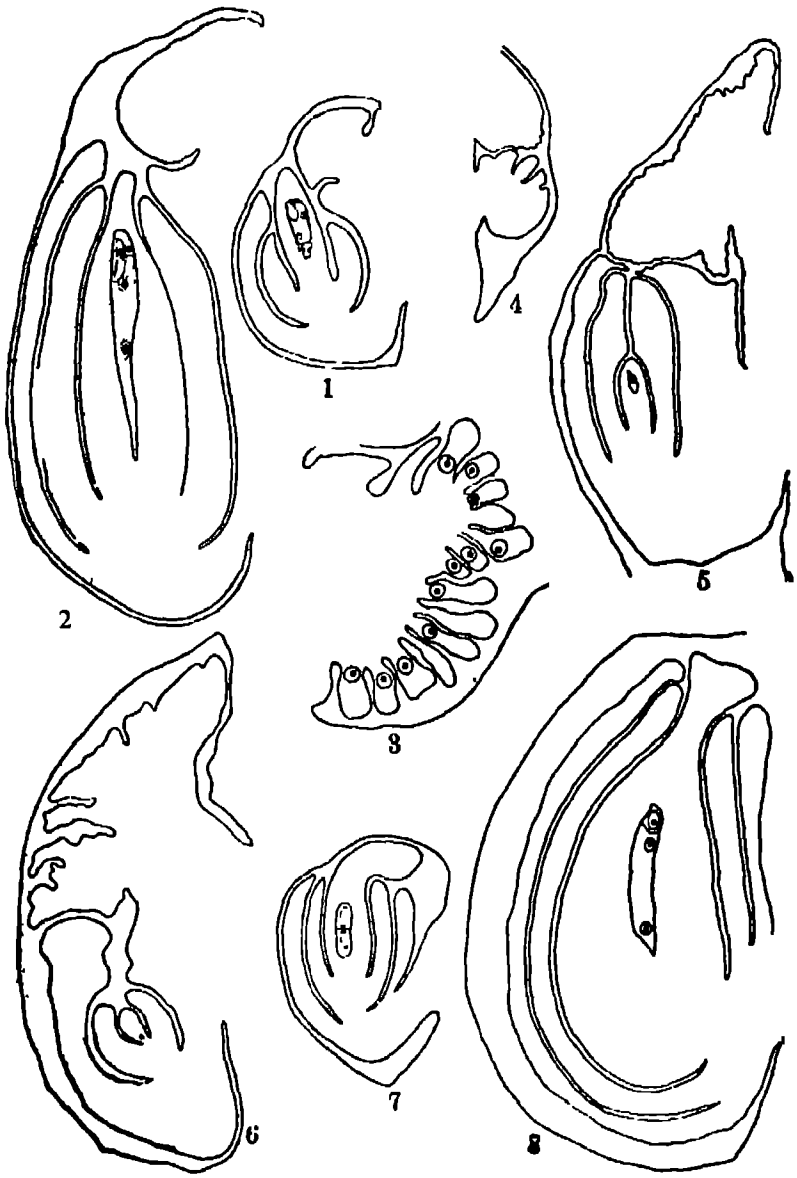
(*b*) *The Nucellus*

In *Putranjiva Roxburghii* and *Trewia nudiflora* the nucellus is comparatively massive and the megaspore mother cell is deep seated At a very early stage of the development of the female gametophyte the nucellus appears to be completely encased by its integuments

Phyllanthus niruri and *Euphorbia thymifolia* are characterized by the presence of nucellar beaks This has been noted previously in the former plant by Maheshwari and Chowdry[7] in 1937. Miss Lyon[5] has noted the presence of a nucellar beak in *Euphorbia corollata*

The "nucellar beak" as observed in the course of the present study shows a difference in its development and orientation. In *Phyllanthus niruri* in the megaspore mother cell stage, the nucellus protrudes out and the integuments just make their appearance as is commonly seen in other plants. Along with the growth of the integuments the nucellus also undergoes marked development and just prior to the reduction division of the megaspore mother cell, the two integuments and the nucellus stand almost at the same level. From this stage onwards, particularly during the second division of the megaspore mother cell the growth of the nucellus appears to be comparatively rapid and it protrudes out in the form of a beak which very soon comes in contact with the ovarian wall and curves towards the placenta as shown in Fig 7. In post-fertilization stages a further growth of the integuments takes place and they lie at the same level as the tip of the nucellar beak (Text-Fig. 8).

In *Euphorbia thymifolia*, protrusion of the nucellar tip becomes evident at t e 2-nucleate stage of the embryo-sac It does not curve but retains its normal orientation even in the mature ovule, where it is finally enveloped by the integuments, leaving a wide micropyle through which the nucellar beak protrudes slightly (Text-Fig. 2) In *Euphorbia hirta* Kajale and Rao[4] found the nucellar beak to protrude in the form of a long finger-like process.



Text-Figs 1-8 *Euphorbia thymifolia*.—Figs 1 and 2 Stages in the development of the integuments and the obturator × 450 Fig 3 The hair-like uninucleate processes of the obturator × 1540. *Putranjiva Roxburghii* Figs 4 and 5 The development of the obturator. × 155. *Trewia nudiflora*. Fig 6 The position of the integuments and the obturator at the M M. C. stage × 155 *Phyllanthus niruri* Fig 7 The position of the integuments and the nucellar beak during the 2nd division of the M M C × 450 Fig 8 The position of the integuments and their nucellar beak at the mature E S. and post-fertilization stages. × 450.

(*c*) *The Obturator*

The presence of an obturator has been recorded in some genera only, such as *Euphorbia*, *Croton* and *Acalypha*. In the present study it was observed that an obturator is present in *Putranjiva Roxburghii*, *Trewia nudiflora* and *Euphorbia thymifolia*. Except in the last, the obturator is fairly well developed even at the megaspore mother cell stage (Text-Figs. 4 and 6). In *Euphorbia thymifolia*, however, only a slight protrusion from the placenta occurs at this stage The later development of the obturator shows no marked difference It results in the formation of a hump-like structure which lies above the micropyle (Text-Figs. 1 and 2). It is interesting to note, however, that in *Euphorbia thymifolia* the outermost cells composing the obturator grow out in the form of hair-like processes and are uni-nucleate (Text-Fig. 3). Kajale and Rao[4] have noted the presence of an obturator of the loose type in the species studied by them. On *Putranjiva Roxburghii* and *Trewia nudiflora* the obturator becomes very massive during the later stages of the development of the female gametophyte and comes to lie just above the micropyle, so that the pollen tube has to follow a circuitous route to reach the embryo-sac (Text-Figs. 5 and 6)

Maheshwari and Chowdry[7] report the presence of an obturator in *Phyllanthus niruri* The present study, however, failed to reveal its presence. It is probable that they mistook the nucellar beak of the second ovule for an obturator. In this connection, some preparations of *Phyllanthus urinaria* were also examined and here too the absence of an obturator was noted. There were two ovules in each loculus and the nucellar beak was very prominent. In longitudinal sections, the nucellar beak of the second ovule sometimes gave the appearance of an obturator but close examination always revealed its true nature

(*d*) *The Development of the Macrospores*

The presence of a multicellular archesporium has been noted previously in various species of *Euphorbia* The present study, however, shows that in *Euphorbia thymifolia* a single archesporial cell occurs in the hypodermal layer of the nucellus and by its division gives rise to the megaspore mother cell and a cover cell (Text-Fig. 9). In *Putranjiva Roxburghii* a multicellular archesporium has been observed from which a single megaspore mother cell develops and is first noted in the 4th layer of the nucellus (Text-Fig. 10), while in *Trewia nudiflora* it generally occurs in the 11th layer and in *Phyllanthus niruri* in the 3rd layer. It might be, as Maheshwari and Chowdry[7] suggest, that the archesporial cell cuts off a parietal layer and then functions as the megaspore mother cell. The megaspore mother cell of *Phyllanthus*

niruri in the later stages of its development is pushed considerably inside the nucellus on account of the rapid divisions of the overlying cells and comes to lie in the 9th or 10th layer.

The megaspore mother cell increases in size before signs of activity. Stages in the reduction division have been observed in all plants studied and show no unusual features On the completion of the first division a dyad is produced These cells soon divide and produce the macrospores, which in every case are arranged in a linear order (Text-Figs. 11, 13, 14 and 15). In *Euphorbia thymifolia*, however, in addition to the linear tetrad of macrospores, T-shaped tetrads have also been observed (Text-Fig 12) In a few cases, the two upper macrospores are somewhat obliquely oriented Maheshwari and Chowdry's investigations on the gametophytic development of *Phyllanthus niruri* shows that " it (megaspore mother cell) undergoes the usual reduction divisions and produces a tetrad of megaspores or a row of three cells of which the lower two are megaspores and the upper an undivided dyad cell " A critical examination of a number of preparations showing this stage failed to corroborate their account.

The lowermost or the chalazal megaspore alone functions while the three upper degenerate in every instance (Text-Figs 11, 13 and 15). The course of degeneration appears to be from above downwards In preparations showing later stages, the degenerated products could be seen as dark shapeless masses capping the functional megaspores

(*e*) *The Development of the Female Gametophyte*

The functional megaspore increases in size and the cytoplasm becomes vacuolated, the vacuoles appearing mostly towards the periphery It is interesting to note that in *Euphorbia thymifolia* two well-defined vacuoles occur above and below the nucleus which is placed centrally.

The binucleate stages are normal but a single instance of abnormality was observed in *Trewia nudiflora* where during the division of the two nuclei the sac was found to have elongated considerably measuring approximately 96·25 μ in length (Text-Fig. 16), its normal length at this stage being approximately 36·26 μ. By two successive divisions of the two nuclei an 8-nucleate embryo-sac is produced, four nuclei being oriented at each of the two ends of the embryo-sac In *Putranjiva Roxburghii*, at the 8-nucleate stage, the nuclei at the chalazal end lie in a linear order, the uppermost one which functions as a polar fusion nucleus being alone separated by a wall (Text-Fig 22). A similar separation of the nuclei destined to be the antipodal cells has also been observed, in *Phyllanthus niruri* (Text-Fig. 17) and *Euphorbia thymifolia* (Text-Fig. 19).

The mature embryo-sac shows the usual organization with the egg apparatus, the polar fusion nuclei and the three antipodals. These are differences, however, in certain details and as such it is proposed to describe each of them separately.

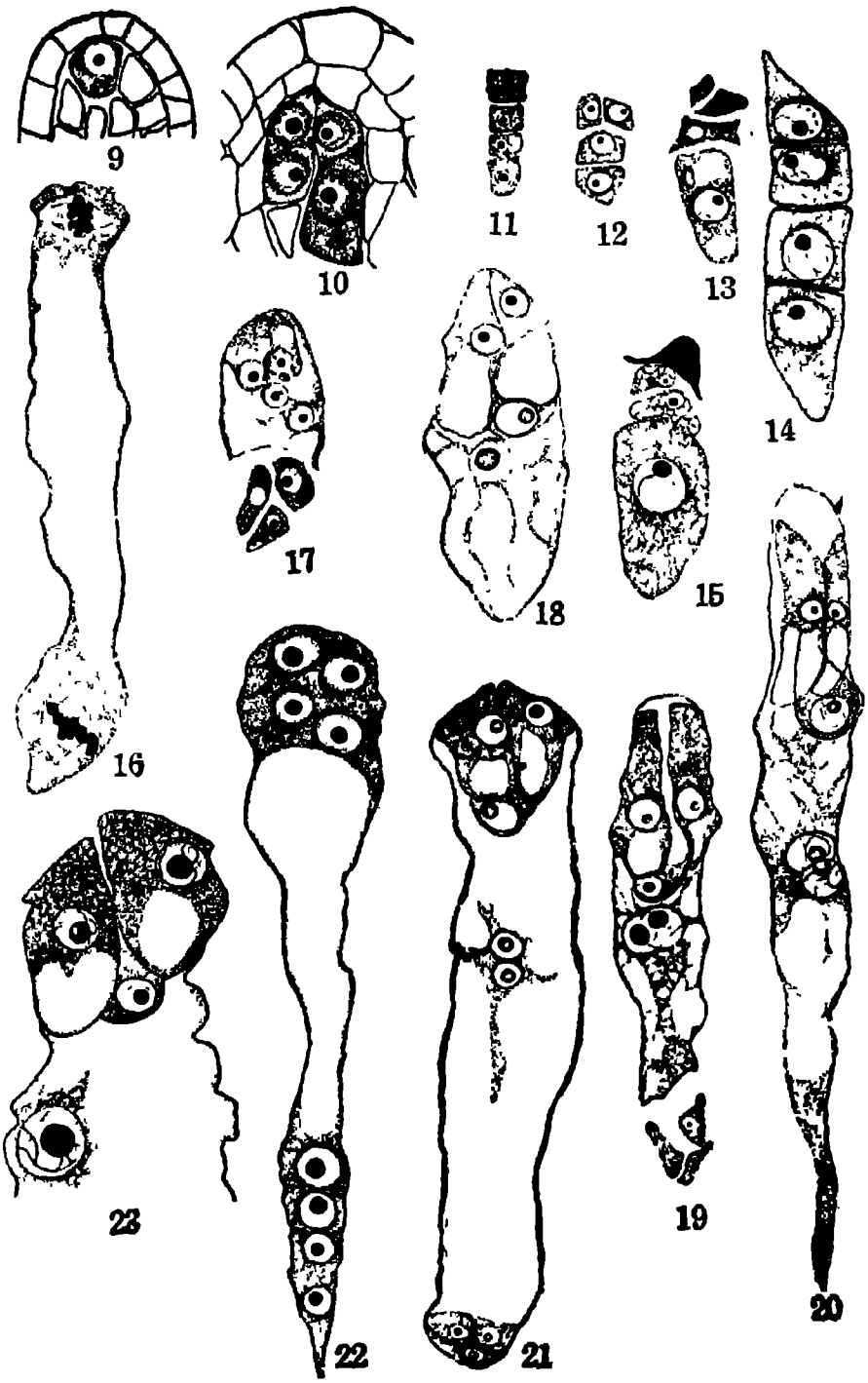
(*a*) *Putranjiva Roxburghii* Wall —The mature embryo-sac is very much elongated approximating 192·5 μ in length The synergids are distinctly hooked with the nuclei placed near the central region and above the large vacuoles which lie towards the base The tip of the synergids shows the presence of innumerable small vacuoles giving the appearance of a honey-comb-like structure. The egg lies below and between the synergids with a vacuole at the top and nucleus embedded in a dense cytoplasm at the base The secondry nucleus appears to be comparatively large lying somewhat close to the egg apparatus and embedded in a dense cytoplasmic mass The antipodals have cytoplasmic walls and are generally arranged one above the other in a linear row They do not show any signs of degeneration when the gametophyte is fully mature (Text-Figs 23)

(*b*) *Trewia nudiflora* Linn —The mature embryo-sac measures approximately 148·5 μ in length. As in *Putranjiva Roxburghii*, the synergids are hook-shaped and the egg apparatus shows no unusual features. The two polar fusion nuclei lie very close to each other near the central region of the embryo-sac, but have not been observed to fuse. The antipodal cells occupy the chalazal end of the embryo-sac (Text-Fig 21).

(*c*) *Phyllanthus niruri* Linn.—The mature embryo-sac measures approximately 45·0 μ in length and appears to be 4-nucleate on account of the degeneration of the three antipodal cells which takes place earlier (Text-Fig. 18). The synergids are slightly hook-shaped and have the usual organization. Critical examination of a number of preparations shows that the egg always lies below one of the synergids and is not centrally placed. The secondary nucleus lies very close to the egg, its nucleus being comparatively large.

It should be pointed out in this connection that after the degeneration of the antipodal cells, the embryo-sac increases considerably in size and the chalazal end of the sac rounds off giving no indication whatsoever of the presence of the antipodals. Thus Arnoldi[1] who had failed to observe the earlier stages regarded it as a monosporic 4-nucleate type.

(*d*) *Euphorbia thymifolia* Burm.— The mature embryo-sac measures approximately 126·5 μ in length. The synergids in this case are somewhat elongated with blunt tips. The egg apparatus shows the usual arrangement of the nuclei and the vacuoles. In all the preparations observed the polar fusion nuclei appear in a state of fusion The antipodals are cut off



Text-Figs 9–24. Explanation in text *Euphorbia thymifolia*.—Figs. 9, 12, 13, 19 and 20. *Putranjiva Roxburghii*. Figs 10, 14, 22 and 23. *Trewia nudiflora* Figs. 15, 16 and 21. *Phyllanthus niruri*. Figs. 11, 17 and 18 Fig 21 × 950; the rest × 1540.

as separate cells (Text-Fig. 19). After their degeneration the embryo-sac elongates further (Text-fig. 20).

Summary

This paper gives a comparative account of the development of the ovules, macrospores and the female gametophyte of some members of the *Euphorbiaceæ, viz., Putranjiva Roxburghii, Trewia nudiflora, Phyllanthus niruri,* and *Euphorbia thymifolia.*

1. The ovules are anatropous and bitegmic

2. In *Putranjiva Roxburghii* and *Trewia nudiflora* the integuments take part in the formation of the long micropyle whereas in *Phyllanthus niruri* and *Euphorbia thymifolia* no true micropyle is formed as the ' nucellar beak ' lies in between the integuments.

3. Nucellar beaks have been noted in *Phyllanthus niruri* and *Euphorbia thymifolia.* In the former plant the beak remains extruded and curves towards the placental surface before the development of the female gametophyte, while in the latter it retains its original erect position from the beginning.

4. The presence of an obturator is reported in *Putranjiva Roxburghii, Trewia nudiflora* and *Euphorbia thymifolia.* The obturator is massive. The outermost cells composing the obturator of *Euphorbia thymifolia* are hair-like and are uninucleate.

5. The single hypodermal archesporial cell gives rise by division to a megaspore mother cell and a cover cell in *Eurphobia thymifolia. A* multicellular archesporium has been noted in *Putranjiva Roxburghii* from which a single megaspore mother cell is formed. The megaspore mother cell is deep-seated in *Putranjiva Roxburghii, Trewia nudiflora and Phyllanthus niruri.*

6. A linear tetrad of macrospores has been observed in all the cases. In *Euphorbia thymifolia* T-shaped tetrads have also been observed.

7. In all the plants the chalazal megaspore alone functions, the others degenerating from above downwards.

8. The development of the embryo-sac is of the normal type. In *Phyllanthus niruri* the mature embryo-sac is 4-nucleate while in *Euphorbia thymifolia* it is 5-nucleate.

9. Hooked synergids have been observed in *Putranjiva Roxburghii, Trewia nudiflora* and *Phyllanthus niruri.* In *Euphorbia thymifolia,* it is somewhat elongated with blunt ends. Secondary nucleus was observed

in *Putranjiva Roxburghii* and *Phyllanthus niruri* In *Trewia nudiflora* and *Euphorbia thymifolia* the polar nuclei remain close to each other but have not been observed to fuse. Antipodals are ephemeral in *Phyllanthus niruri* and *Euphorbia thymifolia*. In *Putranjiva Roxburghii* and *Trewia nudiflora* they persist.

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THE DRY AND WET METHOD OF PLANTING SUGARCANE AND THE STUDY OF SEASONAL CYCLES OF SOME CHEMICAL CONSTITUENTS IN RELATION TO THEM

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Introduction

Sugarcane occupies an area of about 30,000 acres in the Central Provinces and Berar of which about 45 per cent. is in the Chhatisgarh Division. The soils associated with it are locally known as 'kachhar' which is of alluvial nature and kanhar which is a stiff clay resembling black cotton soils of Berar. The chief limiting factor for sugarcane extension in this tract is limited resources of irrigation. The big irrigation works are mainly used for protecting paddy crop from failure of monsoon in September, and sugarcane occupies only a secondary position Sugarcane as is grown at present with canal, tank or well irrigation requires about 20 irrigations with a frequency of ten days. This is known as wet method of planting and involves a great waste of water. An alternative method known as dry method of planting adopted on the lines of that practised in the United Provinces and requiring only 8-10 irrigations was tried with success.[1] This effects a great saving of water and would enable to irrigate nearly double the area with the same amount of water and would especially be useful where sugarcane is grown with lift irrigation.

The investigations concerning important chemical constituents of sugarcane were undertaken to see if any marked differences exist and could be associated with particular method of planting. The work relating to seasonal cycles of total sugars, sucrose, invert sugar and sucrose invert sugar ratio were included to provide a fairly complete picture of these constituents concerned with gur and sugar making processes. These already seem to be of importance in solving the problems relating to gur and sugar making.[1]

Different Methods of Planting Sugarcane

The two methods of planting cane :

(*a*) Wet or irrigated cane,

and (*b*) Dry or sparsely irrigated cane, are described in brief below.

(*a*) *Wet or irrigated cane.*—The method is practised wherever irrigation by canal, tank or well is possible The land is irrigated, ploughed and cross-ploughed and made into ridges and furrows. At the time of planting in January or February irrigation water is allowed to run in the furrows and saturate the soil. Whole canes are stripped by hand and cut into sets of 1'-1½' length having three buds each These are planted in furrows with eyebuds facing on sides and pressed under feet. The germination is uniform and good As the crop is planted near the surface about 15 irrigations with a frequency of ten days are required before the break of monsoon. Two to three hand weedings and 6-8 hoeings have to be given due to abundant growth of weeds. Manuring and earthing are done before monsoon. Subsequent operations are tying of cane and irrigation whenever necessary. An average crop gives about 40,000 lbs. of cane or 50 maunds of gur per acre..

(*b*) *Dry or sparcely irrigated cane.*--In this method of planting a preliminary cultivation is given in November with or without irrigation as conditions would permit One irrigation is given two to three days before planting in December or January and as soon as the land is workable furrows are opened by a ridging plough, sets planted in them and covered with a spade. A planker is then worked twice to level the land and a loose mulch is spread by means of a spike-tooth harrow or a *datari* to conserve moisture. Germination is even and satisfactory. No further irrigation is necessary for about two to three months after planting by which time sugarcane establishes fully. A planet junior hoe is worked along the lines and one irrigation is given every month till the beginning of rains. Manurial and other treatments are the same as in the other method. This method allows frequent hoeings and hence the number of hand weeding is only one

Experimental

Site, Soil and Lay-out —An experiment was carried out at the Government Seed and Demonstration Farm, Bilaspur, during the years 1936-1940 inclusive. The site is practically level and open. The experiment was conducted on one site in the 1st, 3rd and 5th year and on another site close by in the 2nd and 4th year. The soil is of alluvial nature locally known as 'kachhar' and is representative of the tract in which the method is being

advocated. Eighteen plots of 1/40th acre measuring 33' × 33' were laid out in a line and the treatments were allotted as follows:

AXBB X AA X BB X AA X BB XA

Where A—represented wet method of planting,

B—dry method of planting,

and X—represented buffer plot to prevent seepage of water from wet planted to dry planted plots.

The variety used was Co 210 which is a midlate cane.

Management.—The plots were planted as per methods described above. Manure was given at the rate of 150 lbs. of Nitrogen per acre—50 lbs. nitrogen as green manure of Sannhemp at the time of sowing, 60 lbs. nitrogen as oilcake and ammonium sulphate by the end of March and 40 lbs. nitrogen as oilcake and ammonium sulphate by the end of June before earthing. The standard of cultivation throughout was always satisfactory. Other routine practices in sugarcane cultivation were followed.

Chemical investigations.—The chemical data utilized in the present instance have been collected by the staff of the Chemistry Section of the Department of Agriculture, Central Provinces and Berar, Nagpur. The important data available for study was fortnightly record of the weight of individual cane in lb, percentage of juice to cane and grams of sucrose and invert sugar per 100 c.c. of cane juice from first fortnight of November to the second fortnight of April for all the five years 1936—40.

Results

The records collected throughout the experiment related to germination, general growth characters, number of weedings and irrigations given, crop yields, cost of cultivation and net profit. The data for yields of cane and gur for five seasons 1936–40 are given in Appendix A.

Fortnightly samples were taken during the cane crushing period, *i.e.*, November to April for five seasons 1936–40 and weight of individual cane in lb. and percentage of juice as extracted from stripped cane without tops were determined. Estimates of sucrose and invert sugar were made on the juice that was extracted and total sugars and sucrose-invert sugar ratio were calculated. In order to facilitate interpretation, the results of analysis given in Appendix B are presented graphically in Figs. 1–6. The values for each constituent are means for the five years.

Discussion

Two aspects of the data require discussion, *viz.*, (*a*) comparison between dry method and wet method of planting sugarcane and (*b*) seasonal cycle studies.

(a) *Comparison between dry method and wet method of planting.*—The results obtained are classified into three subheads for discussion:

(i) Cultural observations ; (ii) Crop yields and (iii) Chemical data. The data has been analysed statistically and the standard of significance used has been that the chances against a result being fortuitous must be at least hundred to one.[3] To simplify the discussion, the results are arranged in a tabular form in Table I which shows comparision between the two methods of planting.

TABLE I

	Dry method of planting	Wet method of planting	Remarks
(i) Cultural observations –			
(1) Germination	Satisfactory 4–5 days late	Satisfactory	
(2) Number of weedings and hoeings	One hand weeding and 8 -10 hoeings	2–3 hand weedings and 6–8 hoeings	
(3) Number of Irrigations	8 (6 before and 2 after rains)	20 (15 before and 5 after rains)	
(ii) Crop yields –			
Yield of cane per acre	61,302 lb	56,963 lb	Average of five years 1936–40. S E 1379·6 lb
Yield of gur	5,984 lb	5,336 lb	do S.E. 132·8 lb
Cane to gur ratio	10·24	10·68	
Cost of cultivation	Rs 138– 5–0	Rs 145–14–0	Average of four years 1936–39
Value of out-turn per acre	Rs 300–13–0	Rs 268– 4–0	do
Net profit	Rs 162– 8–0	Rs 122– 6–0	do
(iii) Chemical data–			
Weight of individual cane in lb	1·56 lb	1·46 lb	Differences not significant
P C of juice in cane	60·14	60·18	
P C of sucrose in juice	15·93	16·08	
P C of invert sugar in juice	1·50	1·56	
P C. of total sugars in juice	17·43	17·64	
Sucrose/Invert sugar	10·6	10·0	

N B—The data for (i) and (ii) are compiled from the cultivation registers maintained on the Government Demonstration Farm, Bilaspur.

(i) *Cultural observations.*—Germination was found to be satisfactory in both the methods but in the dry method of planting, it was delayed by about 4 or 5 days which may be due to deeper sowing. Late planting is not conducive to germination under dry method of planting and may be

attributed to high temperature coupled with less moisture. This method allows of interculture from the very beginning and hence the number of hand weedings could be reduced to one. Tillering is also promoted due to looseness of soil. The cultivation in general is more clean and the cane crop can utilise the manure with advantage. Number of irrigations are eight in all, six before and two after the rains. In wet method of planting, there is more loss of moisture due to evaporation and 15 irrigations with a frequency of ten days are necessary during summer and five irrigations are required after the rains. Deeper sowing in dry method of planting facilitates earthing operation and enables the cane to withstand ordinary stormy weather. The height and thickness of the crop are about the same in both the cases.

TABLE II

Analysis of variance for the yields of gur in lbs. per 1/40 *acre plot*

Due to	D F	Sum of squares	Mean square	F	Level of significance F		Remarks
					5%	1%	
Blocks	25	12,750					
Seasons	4	12,415	3,103·8	9·34	2·76	4·18	Significant
Treatments	1	3,998	3,998·0	12·03	4·24	7·77	do.
Season × treatment	4	3,569	892·3	2·68	2·76	4·18	Not significant
Error	25	8,307	332·3				
Total	59	41,039					

TABLE III

Analysis of variance for the yields of sugarcane in lbs. per 1/40 *acre plot*

Due to	D F	Sum of squares	Mean square	F	Level of significance F		Remarks
					5%	1%	
Blocks	25	10,94,835					
Seasons	4	21,63,832	5,40,958	15 1	2 76	4 18	Significant
Treatments	1	2,49,667	2,49,667	6·9	4·24	7·77	do at 5%
Season × treatment	4	3,19,500	79,875	2·2	2 76	4 18	Not significant
Error	25	8,92,533	35,701				
Total	59	47,20,367					

(ii) *Crop yields.*—The yield of cane and gur is higher in dry method of planting and the difference is significant. The higher yield may

be due to more tillering and better utilisation of manure due to less competition from weeds. Cane to gur ratio is the same in both the cases. Cost of cultivation is slightly higher in wet method of planting on account of more hand weedings and irrigations. The net profit obtained from dry method over the wet method is Rs. 40-2-0 per acre and is a result of higher out-turn of gur combined with lower cost of cultivation. Dry method of planting cane is, therefore, more economic and profitable than wet method.

(iii) *Chemical data.*—The individual constituents like weight of individual cane, percentage of juice in cane, percentage of total sugars, sucrose and invert sugar in juice and sucrose invert sugar ratio do not show any significant difference indicating that the composition of cane is not affected by the method of planting.

(*b*) *Cycles of individual constituents*

(i) *Average Weight of Individual cane* —The weight of individual cane remains practically constant at 1·56 lb. from November to February. It is low in March and shows a definite fall to 1·37 lb in April. This is due to drying of cane in summer months and agrees closely with the fall in percentage of juice extracted from stripped cane without tops (Fig. 1).

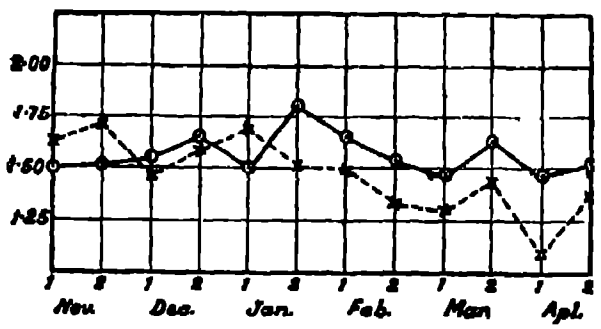

FIG 1 Wt. of individual cane in lbs

(ii) *Percentage of juice in stripped cane without tops.*—The juice content is 72·5% in young mature cane and is at its maximum in the second fortnight of November. It gradually falls down during the following months and reaches to a minimum of 56·5% in March and April (Fig. 2). It is observed that the canes are more juicy in earlier months and the extraction of juice is more complete. In the latter months canes generally dry and become hard and there is some loss in the extraction of juice.

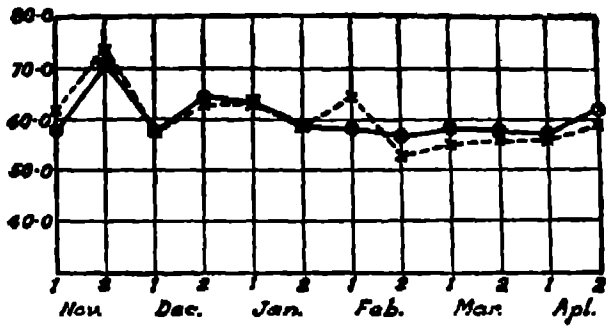

FIG. 2. P.C. of juice to stripped cane without tops

(iii) *Total sugars in juice.*—The total sugar content is obtained by adding sucrose and invert sugar and is shown in Fig. 3. It is as low as 13·1% in the first fortnight of November and rises gradually to a peak of 19·87% in the first fortnight of March. During April the sugar content falls rapidly to 16·3%. Co 210 is a midlate cane and has high percentage of total sugars from December to middle of March which agrees closely with the best gur and sugar making period. In November the extraction of juice is satisfactory but sugar content being less more time is required to boil the juice and make gur out of it During April the percentage of juice extracted is less and the total sugar content also falls down rapidly thus entailing loss in gur and sugar output

(iv) *Percentage of sucrose in juice.*—Sucrose content in the juice is about 10·27% in the first fortnight of November. It rises steadily during the following months and reaches a maximum of 19·11% in the first fortnight of March (Fig. 3). There is a rapid fall in sucrose content in the following month and a half and it reaches 14·48% in the second fortnight of April. The quality of gur depends largely on sucrose content. Gur prepared between December and middle of March has good grain and golden colour, while that prepared in April is pasty, dark brown and does not solidify soon. The best period for sugar-making is from December to first fortnight of March as sucrose content is high during that period.

(v) *Percentage of invert sugar in juice.*—Invert sugar is highest in the first fortinight of November and is about 2 87% It shows a significant fall during the cane crushing period and reaches a minimum of 0·76% in the first fortnight of March. It then rises rapidly during April to a high value of 1·82% (Fig. 4). High percentage of invert sugar affects the colour and grain of gur adversely. It is useful to examine the invert sugar contents in relation to those for sucrose. The peak value for sucrose in the first fortnight of March corresponds to a minimum value for invert sugar.

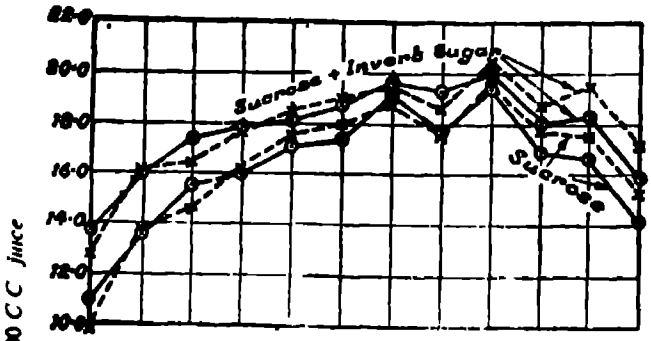

FIG. 3

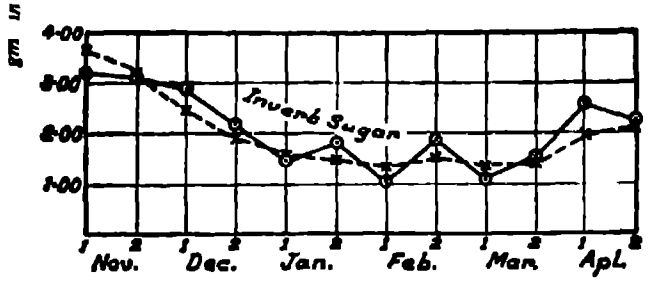

FIG 4

⊙—⊙ Dry method of planting ×—× Wet method of planting

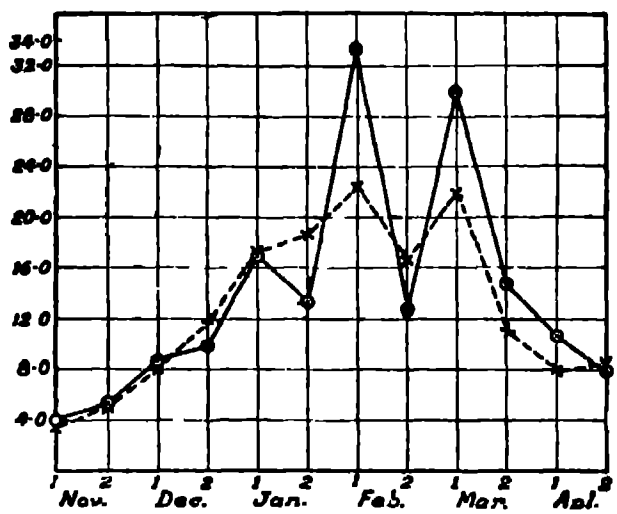

FIG 5 Ratio of Sucrose/Invert Sugar

⊙—⊙ Dry method of planting ×—× Wet method of planting

Curves for invert sugar and sucrose expressed as percentage of juice appear in a general way as reciprocals of one another. There is a very high negative correlation between them which suggests transformation of one into the

other. In earlier months glucose is converted into sucrose and in later months sucrose is inverted to glucose.

(vi) *Sucrose/invert sugar ratio.*—It is lowest in the first fortnight of November and is about 3 55. It then increases rapidly with the rise in sucrose content and fall in glucose content and reaches a maximum of 27·8 in the first fortnight of February and remains at that till the first fortnight of March. It then falls rapidly to 7·9 in April (Fig. 5) A wider ratio of sucrose-invert sugar is more favourable for gur and sugar making.

Conclusions

The main conclusions which may be drawn from the data obtained in the investigations are as follows

(1) *Dry method of planting compared with wet method of planting sugarcane.*

Cultural observations.—Germination is satisfactory in both the methods but it is late by 4–5 days in dry method of planting. Late planting should be avoided in dry method as it results in defective germination.

The dry method allows interculture from the beginning and hence the number of weedings are reduced to one only. The cultivation in general is more clean. The number of irrigations are less than half and hence double the area could be managed by dry method of planting Tillering is promoted by this method.

Crop yields.—The yields per acre of sugarcane and gur are significantly higher in the dry method of planting. Cane/gur ratio is about the same. The cost of cultivation per acre is less due to less number of weedings and irrigations.

The net profit per acre is definitely more in the dry method of planting and the method is, therefore, more economic and profitable.

Chemical data.—There is no difference in the chemical composition of sugarcane grown under the two methods.

(2) *Seasonal cycles of individual constituents.*

Well-defined cycles occur for individual weight of canes, percentage of juice in cane, percentage of sucrose, invert sugar and total sugars in juice and sucrose-invert sugar ratio. The main features of the cycles for these may be summarised as follows

(i) The weight of cane remains constant at 1·56 lb. from November to February and then shows a fall in March and a distinct fall in April to 1·37 lb.

(ii) The percentage of juice in cane ranges from 72·5 to 56·5. It is highest in November and lowest in March and April.

(iii) Total sugars range from 19·87 to 13·1 % of juice. It is low in November, increases steadily to a maximum in the first fortnight of March and later falls in April.

(iv) *Percentage of sucrose.*—The sucrose content in juice ranges from 19·11% to 10·27%. It is low in November, rises steadily to a maximum in first fortnight of March and later falls in April.

(v) *Invert sugar.*—The invert sugar content in juice ranges from 2·75% to 0·76%. It is at a maximum in November, then falls to a lower level and reaches a minimum 0·76% in the first fortnight of March and then rises rapidly again in April There is a very high negative correlation between sucrose and invert sugar content showing transformation of one into the other at different stages in the cycle.

(vi) *Sucrose-invert sugar ratio.*—It ranges from 3·55 to 27 8. It is low in November, rises to a peak value in the first fortnight of March and then falls rapidly in April

These results agree closely with the general experience that best time for cane crushing is between December and mid of March The sucrose content is at its highest and the gur and sugar output are maximum in the first fortnight of March. Gur during this period is crystalline and possesses golden colour. In November the juice extraction is satisfactory but requires more time and labour to boil the juice and make gur. In April the percentage of juice extracted is less and due to high temperature and high invert sugar content the quality of gur deteriorates It is dark brown in colour and solidifies with some difficulty. Wider sucrose invert sugar ratio is more conducive to gur and sugar making.

Summary

(1) An experiment is described in which dry method and wet method of planting sugarcane have been discussed

(2) The seasonal cycles of individual constituents of cane were included in order to determine if any marked differences exist and could be associated with particular method of planting

(3) The experiment was laid out at the Government Demonstration Farm, Bilaspur, during 1936-40 and data regarding cultivation, general growth and crop yields were recorded. Samples of whole cane were collected at fortnightly intervals from November-April for all the five

years and analysed by the staff of the Chemistry Section of Department of Agriculture, Central Provinces and Berar.

(4) The cultural observations made were germination, number of weedings, number of irrigations, tillering, yield of cane and gur, cost of cultivation and net profit. The chemical data collected was weight of individual cane, percentage of juice in cane, percentage of sucrose, invert sugar, total sugar in juice and sucrose-invert sugar ratio.

(5) The cultural observations showed that dry method of planting was more economic and profitable than wet method of planting sugarcane.

(6) The chemical data did not exhibit any difference in the composition of the cane grown by two methods.

(7) The chief constituents determined were found to exhibit definite seasonal cycles and are of importance in diagnosing the practical problems concerning gur and sugar making.

(8) The sucrose and invert sugar contents show a very high negative correlation showing transformation from one to the other form. Wider sucrose-invert sugar ratio is more favourable for gur and sugar making.

Acknowledgment

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STUDIES ON THE HELMINTH PARASITES OF KASHMIR

Part III. Description of a New Allocreadid, *Crepidostomum indicum*, from a fresh-water fish, *Schizothorax niger*, from the Dal Lake, Kashmir

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The genus *Crepidostomum* was erected by Braun (1900) and was placed under the sub-family Bunoderinæ by Looss (1902) and under Stephanophialinæ by Nicoll (1909) Subsequently several other genera were described under papillose Allocreadiidæ, but the recent researches of Hopkins (1931, 1933 and 1934), Van Cleave and Mueller (1934) and Lyster (1939) have reduced their number to three, *viz.*, *Bunodera* Raillet (1896), *Crepidostomum* Braun (1900) and *Creptotrema* Travassos, Artigas and Pereira (1928)—*Acrodactyla* Stafford (1904), *Stephanophiala* Nicoll (1909), *Acrolichanus* Ward (1918) and *Megalogonia* Surber (1929) being considered synonyms to *Crepidostomum*. Besides, Hopkins (1933 and 1934) and Van Cleave and Mueller (1934) established a close relationship of *Crepidostomum* with *Bunodera* and *Allocreadium* and found no justification for maintaining the sub-family Stephanophialinæ of Nicoll (1909) and the family Bunoderidæ of Poche (1926) and Fuhrmann (1928).

There are twenty-two species reported under the genus *Crepidostomum* but Van Cleave and Mueller (1932 and 1934) and Hopkins (1933 and 1934) have reduced their number to the following eleven species:—

1. *C. farionis* Müller (1788). Syn. *Stephanophiala laureata* Zeder (1800), *S. transmarinum* Nicoll (1909), *S. vitelloba* Faust (1918), *C. ussuriense* Layman (1930) and *C. fausti* Hunnien and Hunter (1933);

2. *Crepidostomum* (*Distomum*) *auriculatum* Wedl. (1857);

3. *C. metæcus* Braun (1900), Syn. *C. suecicum* Nybelin (1932),

4. *C. lintoni* Pratt (Linton, 1901). Syn. *Acrolichanus* (*Acrodactyla* Stafford, 1904) *petalosa* Looss (1902);

5. *C. cornutum* Osborn (1903);

6. *C. illinoiense* Faust (1918), Syn. *C. hiodontis* Hunter and Bengham (1932);

7. *Crepidostomum* (Syn. *Megalogonia*) *ictaluri* Surber (1928);

8. *C. latum* Pigulewsky (1931);

9. *C. cooperi* Hopkins (1931), Syn *C amblopliti*s Hopkins (1931), *C. solidium* Van Cleave and Mueller (1932);

10. *C. isostomum* Hopkins (1931), Syn. *C canadense* Hopkins (1931);

11. *C. brevivitellum* Hopkins (1934)

As will be discussed later in this paper, the author believes that *C auriculatum* and *C lintoni* are also identical and hence the number of valid species would be reduced to *ten*. In the present communication the writer describes another species of the genus obtained from the intestine of a fish, *Schizothorax niger* caught from the Dal Lake in Kashmir. This is the first record of the genus *Crepidostomum* from India

Crepidostomum indicum (n sp.)

Body is more or less flat and lanceolate and measures 2·24 ×0·72 mm. in size, the maximum breadth being in the region of the ovary. There are no body spines. The oral sucker is subterminal, broad and oval, and measures 0·12 × 0·17 mm It bears six preoral papillæ at its anterior end, each being 0·03–0·06 × 0·11–0 13 mm. in size. They are broader than long and each one appears lobe-like, being anteriorly rounded or flat with a silght notch in front. Acetabulum is larger than the oral sucker and lies at a distance of one-fifth of the body-length from the anterior end It is circular with a diameter of 0·27 mm.

Mouth is ventral. Pharynx is globular and greatly muscular with a diameter of 0·13. Oesophagus is narrow and elongated, being three times the length of the pharynx. Intestinal cæca are long and terminate just behind the posterior testis.

Testes are two and lie medially one behind the other in the posterior half of the animal. They are entire, oval and elongated. The two testes are nearly equal, the anterior one measures 0·37 × 0·22 mm. and the posterior one 0·37 × 0·2 mm., in size. Two vasa efferentia unite to form a small vas deferens at the posterior end of the cirrus sac. Cirrus sac is well developed and nearly oval in shape, measuring 0 33 × 0·17 mm. It lies immediately in front of the intestinal fork and contains inside it a coiled vesicula seminalis, pars-prostatica and well-developed cirrus.

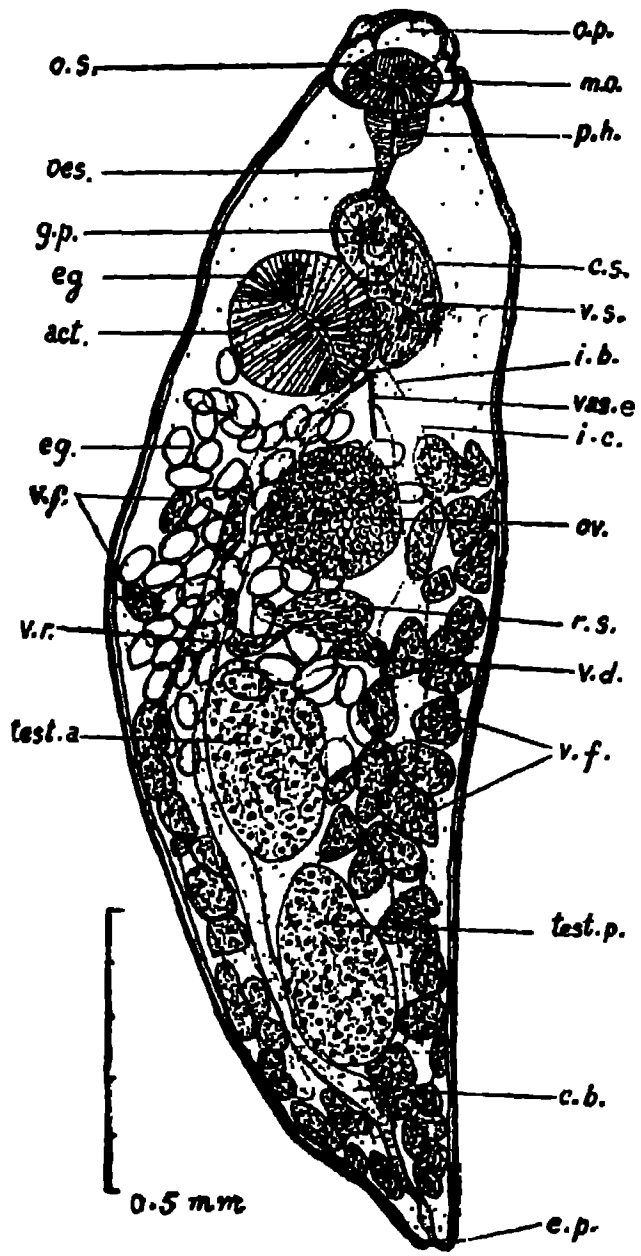

FIG. 1. *Crepidostomum indicum* (n sp) from *Schizothorax niger* (ventral view)

act, acetabulum ; *c s*, cirrus sac ; *eg*, egg ; *e b*, excretory bladder, *e p*, excretory pore ; *g p*., genital pore ; *i.b*, intestinal bifurcation, *i c*, intestinal cæcum, *mo*., mouth, *oes*, oesophagus, *o p*, oral papilla ; *o s*, oral sucker, *ov*, ovary, *ph*, pharynx ; *r s*, receptaculum seminis ; *test.a*., anterior testis, *test p*, posterior testis, *v.d*, vitelline duct; *v.f*, vitelline follicles ; *v.r*., vitelline reservoir ; *v.s*., vesicula seminalis ; *vas.e*., vas efferens.

Ovary is globular and median, measuring 0·24 × 0·23 mm. and lies in the second quarter of the body, behind the intestinal fork and acetabulum.

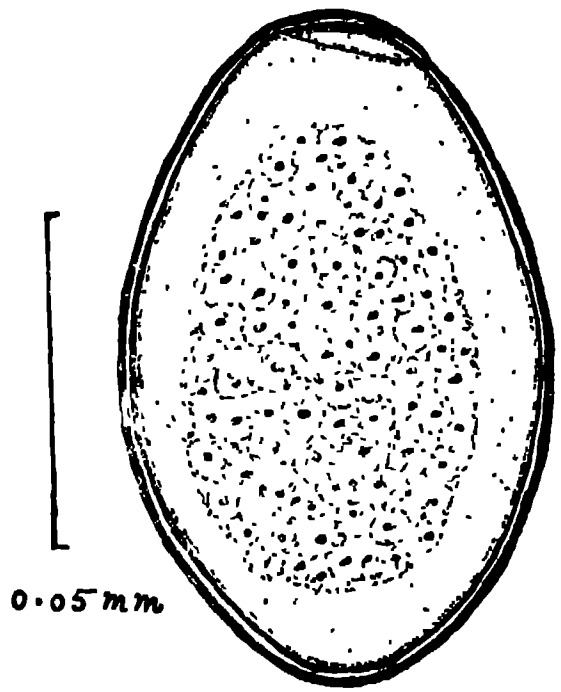

FIG. 2. *Cryptotrematium indicum* egg.

Receptaculum seminis is pear-shaped, measures 0·11 × 0·2 mm. and lies between the ovary and the anterior testis. It receives Laurer's canal before opening into the oviduct. Vitellaria are follicular and the follicles extend laterally from the intestinal fork upto the posterior end of the body, filling up the area behind the posterior testis. They cover the intestinal cæca on the ventral and the lateral sides. Two trans-vitelline ducts meet behind the receptaculum seminis to form a vitelline reservoir. Uterine coils are indistinct and the eggs, numbering 90–100, lie between the acetabulum and the middle region of the anterior testis. They are operculate, nearly oval and large in size, measuring 90–105 × 56–80 μ. Metraterm is not distinct. Genital opening is median and lies in front of acetabulum and intestinal bifurcation.

Excretory pore is terminal and leads internally to form an elongated, tubular and undivided excretory vesicle.

Discussion

The present form differs from *C. metœcus, C. lintoni, C. cornutum, C. illinoiense* and *C cooperi* in the position of genital pore and the anterior extension of vitellaria. It is different from *C. latum* and *C. brevivitellum* in the position of genital pore, from *C farionis* in the anterior extension of vitellaria and from *C ictaluri* in the position of genital pore, anterior extension of vitellaria and form and arrangement of testes. It, however, stands very near to *C isostomum* from which it can be readily distinguished by the shape of its body, notched appearance of the oral papillæ, relative length of œsophagus, form and size of cirrus sac and number and size of eggs The writer, therefore, creates a new species, *C. indicum* for its reception.

Distomum auriculatum Wedl (1857) is inadequately described by its author and according to Stafford (1904) and Faust (1918) the form described and provisionally placed under this species by Linton (1898) is really *Crepidostomum lintoni* (Syn *Acrolichanus petalosa*) Hopkins (1933) while recognising *C auriculatum* Wedl. (1857) as a species distinct from *C. lintoni*, recorded the following difference between the two:—

(*a*) *C lintoni* has oral sucker much larger than acetabulum while *C. auriculatum* has either suckers equal or oral sucker only slightly larger than acetabulum and (*b*) *C lintoni* has uterus reaching behind the anterior testis in older specimens while *C auriculatum* does not show any such condition of uterus

From the above it appears that the points differentiating the two species are variable within the species and hence not valid for specific diagnosis Moreover according to Faust (1918), Hopkins (1933), Van Cleave and Mueller (1932 and 1934) and Lyster (1939 and 1940) the above differences are also reported as instances of individual variations in the other species Sucker ratio is seen to vary in *C farionis, C ictaluri, C. cooperi* and *C. isostomum* and the posterior extent of uterus varies in *C cornutum, C. ictaluri* and *C cooperi*. Thus the two species lack morphological characters to warrant them as separate species and the differences reported fall within the bounds of individual variations.

The present work was carried out at the University of Lucknow under the direction of Dr. G. S. Thapar to whom the author wishes to express his gratitude for guidance and other help received during the progress of this work

Note—Type specimen is deposited in Dr Thapar's Helminthological Collections, Lucknow University.

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THE ACCUMULATION AND MOVEMENT OF NICOTINE IN RECIPROCAL GRAFTS BETWEEN TOBACCO AND TOMATO PLANTS

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Received September 8, 1943

Introduction

In 1934–35, and subsequent crop-years, a large number of grafts was made at the Imperial Agricultural Research Institute in connection with studies on the leaf-curl diseases of tobacco and reported upon by Pal and Tandon.[1] As the grafts included some between tobacco and plants of other species, it was considered a suitable opportunity to study the problem of the accumulation and movement of nicotine. Grafe and Linsbauer[2] had already shown that *Nicotiana affinis* which does not normally possess nicotine in any of its parts, has as much as 1·67 per cent. when grafted on *N Tabacum*. But there was obviously a need for further work on this problem.

Materials and Methods

The tobacco variety used in these experiments was *N. rustica* Type 18, which has a relatively high nicotine content. The Pritchard veriety of tomato was selected as it grows well in Northern India. In the earlier experiments, ordinary cleft grafting was done, the grafts being made reciprocally, *i.e.*, some with tobacco as stock and tomato as scion, and some the other way about. The scions in each case showed wilting in the daytime for about three days but after that regained normal turgidity; new growth was visible after about a week. In the later experiments (1937–38) lateral grafts were made. In 1934–35, the experiment was of a preliminary nature.

In the following crop-year the grafting experiments were repeated on an extended scale, using the same varieties and technique as previously. The tobacco seed was sown and the seedlings transplanted on the 31st August and 2nd October respectively. The grafts were made on 30th November—4th December. Two grafts with tomato scions and tobacco stocks and two of the reciprocals were pulled up for analysis on the 9th December, *i.e.*, ten days after grafting. Thereafter four grafts (two of each reciprocal)

were pulled up every fortnight and analysed. Two non-grafted plants each of tobacco and tomato were pulled up for analysis at the same time as the first batch of grafts.

Nicotine was estimated by the method developed by Wo-sley[8] using freshly prepared lacmoid solution as the indicator. The method is quicker and simpler and gives values agreeing with those of Young's modification of Keller's method.[6]

Experimental

1. Nicotine Content in Non-grafted Plants of Tobacco and Tomato

As mentioned in the preceding section, two plants each of tobacco and tomato were analysed for nicotine content at the same time as the first batch of grafts in 1935-36. The percenatge nicotine based on air-dry samples was as follows.—

	Tobacco		Tomato
	Plant No 1	Plant No. 2	
Roots	0·45	0·24	Nil (or traces)
Lower stem	0·51	0·38	,,
Upper ,,	0·57	0·16	,,
Lower leaves	1·26	0·97	,,
Upper ,,	0·85	0·77	,,
Fruits	..	.	,,

It will be observed that while the alkaloid is present in all parts of the tobacco plants, it is entirely wanting in the tomato plants. In the former it is present to the largest extent in the leaves, the stem and roots containing considerably smaller amounts There is a variation in the nicotine content of the two tobacco plants, one of them, possessing less nicotine than the other in all the plant parts studied

2. Nicotone Content in Grafted Plants of Tobacco and Tomato

(1) 1934–35.—A preliminary experiment was carried out in this year and the results are summarised below.—

Nicotine percentage on air-dry material

Tobacco stock	9·37
Tomato scion	1·27
Tomato stock .	Nil
Tobacco scion . . .	0·35

(2) 1935–36.—The results are given in detail in Tables I and II.

TABLE I

Percentage of Nicotine Content (on air-dry samples) of Grafts where Tomato was Scion and Tobacco was Stock

Date of sampling	Roots	Stem		Leaves		Fruit	
		Old	Young	Old	Young	Green	Ripe
		Tomato Scion					
9–12–35 Pl 1		Nil					.
,, 2 .		,,					
24–12–35 ,, 1		,,			0·87		
,, 2		,,			1·19	..	
8– 1–36 ,, 1		,,			0·35		.
,, 2		0·30		2·99	1·52		
22– 1–36 ,, 1		0·07			2·62		.
,, 2		Nil	.		1·59		.
5– 2–36 ,, 1 .		0·31	.	2·10	1·50		.
,, 2		0 44		2·56	1·37		
19– 2–36 ,, 1		0·15	0·04	1·98	1·22		.
,, 2		0·03	Nil	1·30	1·37		
4– 3–36 ,, 1		Nil		0·81	0·48	.	
,, 2 .		0·04	.	1 46	0·71		
19– 3–36 ,, 1 .		0·04		1·22	0·85		
,, 2		N T *		1·42	0·85	Nil	
11– 4–36 ,, 1		0·01		0·77	0·72	,,	Nil
,, 2 .		0 05	.	1·18	1·19	N T.	N. T.
		Tobacco Stock					
9–12–35 Pl 1 . .	0·81	1·65		4·50			
,, 2	0·97	1·70		4·94			.
24–12–35 ,, 1 .	1·13	1·86	.	6·76			
,, 2 .	1·13	2·43		6·32		.	
8– 1–36 ,, 1	1·22	1·66		8·59			
,, 2	1·18	1·58		7·94			
22– 1–36 ,, 1	0·77	1·18	1·26	8 02	3·16		
,, 2 .	0·85	1·26	1·05	8·42	2 19	.	.
5– 2–36 ,, 1	0·89	1·74	1·56	7·29	2·11		.
,, 2 .	0·61	1·50	1·05	5·83	2·59		
19– 2–36 ,, 1	0·81	1·46		9·15			
,, 2	0·65	0·69	1·38	1·86	1·86		
4– 3–36 ,, 1	0·65	0·73			1·83		
,, 2	0·60	1·01			1·13		
19– 3–36 ,, 1	0·49	0·49		3·20	1·50	0·28	0·57
,, 2	0·59	0·93		0·86	1·49		.
11– 4–36 ,, 1	0·45	0·31					
,, 2	0·53	0·57		3·08	2·87		0·61

* N. T. = nil or traces

Tables I and II demonstrate that whereas in the grafts where the tobacco is used as the stock and the tomato as the scion, the latter distinctly shows the presence of nicotine, in the reciprocal grafts the tomato stock is devoid

TABLE II

Percentage of Nicotine Content (on air-dry samples) of Grafts where Tobacco was Scion and Tomato was Stock

Date of sampling	Roots	Stem		Leaves		Fruit	
		Old	Young	Old	Young	Green	Ripe
		Tobacco Scion					
9-12-35 Pl 1		Nil					
,, 2		,,					
24-12-35 ,, 1		,,			Nil		
,, 2		,,			0·12		
8- 1-36 ,, 1		,,			N T*		
,, 2		,,		Nil	Nil		
22- 1-36 ,, 1		,,			,,		
,, 2		,,			0·07		
5- 2-36 ,, 1		,,		N T	Nil		
,, 2		,,		,,	N T.		
19- 2-36 ,, 1		0 06	0·06	Nil	0·02		
,, 2		0·04	Nil	,,	Nil	Nil	
4- 3-36 ,, 1		Nil			,,	N T	
,, 2		,,			0·03	Nil	
19- 3-36 ,, 1		N T		N T	0·01	,,	0·02
,, 2		Nil		0·06	0·01	0·01	Nil
11- 4-36 ,, 1		0 02		0 03			N T
,, 2		0·04		Nil			Nil
		Tomato Stock					
9-12-35 Pl 1	Nil	Nil		Nil			
,, 2	,,	,,		N T			
24-12-35 ,, 1	,,	,,		Nil			
,, 2	,,	,,		,,			
8- 1-36 ,, 1	,,	,,		N T			
,, 2	,,	,,		Nil			
22- 1-36 ,, 1	,,	,,		,,	,,		
,, 2	N T	,,	Nil	,,	Nil		
5- 2-36 ,, 1	Nil	0 01		,,	,,		
,, 2	,,	Nil		N T			
19- 2-36 ,, 1	,,	,,	Nil	Nil	Nil		
,, 2	,,	,,	,,	,,	,,	,,	
4- 3-36 ,, 1	,,	,,		,,	,,	Nil	
,, 2	,,	,,		,,	,,		
19- 3-36 ,, 1	,,	,,		,,	,,	Nil	
,, 2	,,	,,		N T	,,		
11- 4-36 ,, 1	N T	,,		Nil	N T	N. T.	Nil
,, 2	,,	,,		N T	Nil	Nil	,,

* N T = nil or traces.

of nicotine and the quantity of nicotine in the tobacco scion is very much reduced. The data are discussed briefly below

Tomato scions on tobacco stocks—On examining the results of periodical analyses in Table I it will be seen that in the tomato portion of the graft, there was no nicotine till 24-12-35, *i.e.*, about 25 days after

making the graft, when it appeared in the young tomato leaves. In the older leaves it was first detected in the samples taken on 8–1–36.

In the old tomato stem periodical increases and decreases are to be noted indicating movement of nicotine from the tobacco portion below the graft and possibly transference to the other parts of the plant. This view gets support from the increase in the nicotine content of old leaves and by the appearance of nicotine in the young leaves of the scion (tomato) even as early as the last weak of December

Again, little or no nicotine was found in tomato fruits, but here it is not clear whether nicotine was used up in fruit development or spread over a larger tissue area. It is, however, certain that nicotine was not toxic to the tomato portion of the graft.

Tobacco scions on tomato stocks.—The results of analyses of these grafts detailed in Table II tell a different story. There was no downward movement from tobacco to tomato. Even in the tobacco scion the nicotine is too low to assume distribution over a larger area of the plant. It was probably used up in the elaboration and growth of the tobacco portion above the graft, in the flowering and fruiting processes. This is as would be expected, for as Theron and Cutler[5] observe, nicotine is a storage product which is drawn upon for elaborating material for growth or fruit formation or both. One of us has made similar observations in regard to the growth and development of the seed of *Cannabis indica*, where the oleo-resin is used up in the formation of seed.[6]

The results of the experiments conducted during the period 1934-36 were summarised in a paper read by us before the Indian Science Congress in January 1938.[7] It was then pointed out that "the tomato scions in grafts where tobacco was used as the stock distinctly showed the presence of nicotine suggesting upward translocation of the alkaloid manfactured in the tobacco stock, whereas in the reciprocal grafts the tomato stocks were devoid of nicotine and the quantity of the latter in the tobacco scions was very much reduced". It was also stated that nicotine content increased with maturity in the tobacco plants up to a stage after which there was a decline.

3 *Further Experiments with Grafts*

The experiments were interrupted in 1936 as a result of the transfer of the Imperial Agricultural Research Institute from Pusa to New Delhi. Work on this subject was resumed when in order to study further the translocation of nicotine, a subsidiary experiment was conducted during the winter of 1937–38. In this experiment plants of tobacco and tomato were

grafted together in pairs, the grafting being done laterally (*i e*, a longitudinal piece of the cortex was pared away from the stem of each plant for a distance of about three inches in the middle of the stem, and the two surfaces thus exposed were brought into close contact with each other and tied together in the same way as for other grafts) and both plants being allowed to remain *on their own roots*. When union was well established, after a period of about two months, the constituents of each graft were separated by gently tearing away the fused tissues at the line of union, and grown separately. Analyses for nicotine content were made on samples taken at the time of separation of the grafts and on leaf samples taken three weeks later, from two grafts Leaves of non-grafted tobacco plants were also analysed.

The results are summarised below·—

Tobacco leaves (non-grafted)	Nicotine percentage
1st plant	0·57
2nd plant	0·63

Tobacco leaves from grafted plants	At the time of separation	Three weeks after separation
(*a*) *Above graft*—		
1st plant	0·63	0·22
2nd plant	1·04	0·25
(*b*) *Below graft*—		
1st plant	0·77	No leaves available
2nd plant	0 99	,,

Tomato leaves from grafted plants	At the time of separation	Three weeks after separation
(*a*) *Above graft* -		
1st plant	Nil	0·17
2nd plant	,,	Nil
(*b*) *Below graft*—		
1st plant	Nil	No leaves available
2nd plant	0 45	,,

Although the number of samples is small, it is clear that nicotine or the substances necessary for its synthesis has moved laterally from the tobacco stem into the tomato stem and thence to the tomato leaves. In the case of the first plant nicotine could not be detected in tomato leaves at the time the grafts were separated, but it was present when the leaves were sampled three weeks later. The second plant did not show this, but as the quantities

present at this stage are small it is prossible that traces were actually present but could not be detected The results of analysis of the tomato leaves below the graft in the second graft are anomalous in that this is the only instance where nicotine appears to have travelled downward in the tomato plant

The fact that in the first tomato plant nicotine could not be detected at the time of separation but was found three weeks later appears to indicate that nicotine is not necessarily translocated as such.

Our experiments are not yet completed and further work is contemplated. In view however of an interesting paper by Dawson[8] in which the results of similar experiments are presented and discussed it has been deemed desirable to publish the results obtained so far. A statement showing points of agreement and difference between our results and those of Dawson is given below :—

Dawson's results	Our results
1. When tobacco scions are grown upon tomato stocks no appreciable accumulation of nicotine in tobacco leaves or stems occurs	Do
2. When tomato scions are grown upon tobacco stocks nicotine is found in small quantities in the tomato stems and fruits, and large quantities of the alkaloid accumulate in the leaves	Do, as far as stems and leaves are concerned It was doubtful whether or not fruits contained traces of nicotine
3 Nicotine accumulates only in those grafts which possess a root system	Do "Lateral grafts" (these were not attempted by Dawson) indicate that nicotine may appear sometime after the tomato portion has been separated
4. In tobacco scions on tomato stocks only the basal portion of the scion contained the nicotine, the central and upper portions containing no traces	Young leaves (representing new growth) were analysed separately only once. On this occasion a value of 0·06 was obtained in one plant, the other showing no nicotine A sample of older leaves analysed at the same time showed no nicotine at all
5 Lateral transport of materials across the graft union was found in "approach grafts".	Do in the case of our "lateral grafts"
6. A kind of leaf injury occurs when nicotnie accumulates in tomato leaves	Not observed
7 No evidence was found of downward movement of nicotine	Do, except in one case of a "lateral graft".
8 The author appears to think that nicotine is translocated as such and not as some precursor or intermediate.	The development of nicotine in the tomato constituent of a "lateral graft" only some time after its separation does not support this assumption.

Discussion

Carpenter.[9] Jurits[10] and Chamberlain and Clark[11] have shown that percentage nicotine in the tobacco leaf increases with maturity, while

Cutler *et al.*[13] found that in Turkestan tobacco (*N. rustica*) the nicotine content decreased if the plants were allowed to become over-mature. Earlier experiments by one of us (B. V. N.) have shown that tobacco seed and young seedlings are free from nicotine and that it is developed subsequent to the transplantation of the seedlings and increases until flowering time when it rapidly falls off in the period between flowering.

Statement showing nicotine content in different parts of the tobacco plant, at various stages of its growth:—

Age of plant in days	% nicotine in			Flower and fruit
	Leaf	Stem	Root	
25	Traces			
45	0·48		0·20	
70	1·50	0·22	0·35	
92	2·78	0·57	0·55	
135	4·50	0·51	0·50	
170	3·00	0·31	0·50	
200	1·69	0·20	0·30	Traces

The material was grown in the Guntur area where sowing of seed is done at the end of August, and transplanting of seedlings in early October.

The increase of nicotine in the stem, root and leaves and its eventual decline suggest that it is a reserve product which is used up when the plant matures and begins to form seed. In the case of tomato scions grafted on tobacco stocks there appears to be similar rise and fall in the nicotine content although this is not so clear as in the case of the non-grafted tobacco plant. It would be interesting to follow what actually happens to the nicotine which is accumulated in the tomato scions; in the absence however of experimental data on this aspect of the problem, speculation would be idle and the drawing of conclusions must be deferred until more work on the subject is done.

Summary

1. The distribution of nicotine between stock and scion in reciprocal grafts between tobacco (*N. rustica*) and tomato (var. Pritchard) was studied. The tomato scions in grafts where tobacco was used as the stock distinctly showed the presence of nicotine in the stem and leaves. In the reciprocal grafts however the tomato stocks were devoid of nicotine and the quantity of the latter in the tobacco scions was very small.

2. Besides the cleft grafts referred to in 1 above, a number of lateral grafts were also made, the tomato and tobacco components being separated

FIG 2 A graft with tobacco scion and tomato stock

FIG. 1 A graft with tomato scion and tobacco stock

about two months after grafting and analysed at the time of separation and again three weeks later. In these grafts also there was evidence that nicotine had passed, in this case laterally, from the tobacco to the tomato.

3. Indication was found that nicotine content increases with maturity in tobacco plants up to a certain stage after which there is a decline.

4. The results obtained by Dawson in America are referred to and discussed in the light of the present data The role of nicotine in the metabolism of the tobacco plant is also very briefly discussed.

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OBSERVATIONS ON THE ACCLIMATISATION, NESTING HABITS AND EARLY DEVELOPMENT OF *OSPHRONEMUS GORAMI* (LACÉPÈDE)

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Introduction

Osphronemus gorami (Lacépède), popularly known as Gourami is a native of Malay Archipelago On account of its large size, bonelessness and delicate flavour and the ease with which it breeds, it is considered to be the best fresh-water fish for pisciculture (Sundara Raj, 1939). It has been recently introduced into Europe, Australia, Philippines, India and Ceylon. It is a herbivorous, non-predaceous and hardy fish capable of breeding in confined waters, and is therefore ideally suited for cultivation in ponds. In India it has recently been introduced into several parts of Madras Presidency, Bombay Presidency and Mysore State where it has acclimatised itself and is breeding well. There is every reason to think that if it is cultivated in different parts of India it would make the inland fisheries highly productive It is well known that a study of the breeding habits and mode of development of food fishes is an essential prerequisite for organising fishery development. In this connection the contributions of Sundara Raj (1916), Gopala Aiyar (1935), Jones (1937 *a*, 1940), Job (1941), Hamid Khan (1943) and Kulkarni (1939) on the breeding and development of certain Indian fishes are very helpful in carrying out similar investigations on the food fishes of India. Considerable attention is being paid to the study of the bionomics and development of Gourami by several workers. Roxas and Umali (1937) in an article entitled "Fresh water fish-farming in Philippines" have made cursory observations on a few larval stages of this species Kulkarni (1939) is the first to record the embryonic and larval development of Gourami but his description requires modification and elaboration. The following notes relate to the acclimatisation, nesting habits and early development of Gourami.

Acclimatisation

Eight dozen Gourami fingerlings ranging from 6 to 9 inches in length were purchased from the Madras Fisheries Department in February 1942 and

introduced into a breeding pond at the Markonahalli Fish Farm. There was considerable mortality of the fishes of this lot as they were affected with fin-rot when they were supplied. Some of them survived after they were given a course of saline bath. About a year after introduction there were 25 Gourami, 12 to 15 inches in length.

The Gourami breeding pond of the Fish Farm has a waterspread area of about 1,400 sq. yards. It slopes gradually from the margin to the centre where there is 8 feet depth of water. Before the fishes were let in, the pond was rendered fertile with manure and the introduction of aquatic vegetation, and is now rich in aquatic organisms The fishes are also artificially fed with baked ragi flour mixed with groundnut oil-cake and rice bran. The breeding of Gourami was first noticed in the pond during August 1943.

It is stated in the Service Bulletin No. 3. (Pisciculture) of the Madras Fisheries Department (1939) that " It (Gourami) is not suitable for cold hill water at elevations above 1,000 feet Breeding is satisfactory up to elevation of 1,000 feet with a water temperature of 80° F." The elevation of Markonahalli Fish Farm is 2,400 feet above sea-level and the temperature of water in fish ponds is 75 to 80° F. Here Gourami is not only thriving well but also breeding freely.

Nesting Habits

In an article recently published, Jones has given a history of the previous work on the nesting habit of Gourami Carbonnier (quoted by Jones) describes a floating " bubble nest " made of air bubbles and the buccal secretion of the fish Gilbert (1894) observed eggs of Gourami as having been plastered to the under-side of a rock and supplied with bubbles of air by the parent fish. A reading of the papers of these authors clearly indicates that it is not the nesting habit of the true Gourami (*O. gorami*) that they have described but of some other Anabantoid fishes which are known to construct floating nests of air bubbles. The Editor of the *Journal of Bombay Natural History Society* has rightly pointed out in commenting on Jone's paper that divergent views expressed by various authors is due to the mistake in the identity of the species That Gourami builds a nest of aquatic plants for the reception of its eggs has been fully established by Sundara Raj (1916), Roxas and Umali (1937) and Kulkarni (1939).

The nest of Gourami is constructed out of aquatic plants and is more or less spherical or to be more precise slightly ovoid in shape. The size and form vary slightly from nest to nest. It is slightly thicker in front where there is the opening. The length and width of the nest are about 15 and 12 inches respectively. The size and shape of the nest conforms with those

described by Kulkarni from Bombay. The mouth is circular about 4" in diameter and directed towards the deep portion of the pond. The cavity inside is not deep, being just a shallow depression. The eggs and larvæ are very well protected within the thick nest. In the pond at Markonahalli Fish Farm, the nests are anchored to the long stems and leaves of bulrush (*Typha augustata* B and Ch) Fig. 1 The nests are constructed along the weedy

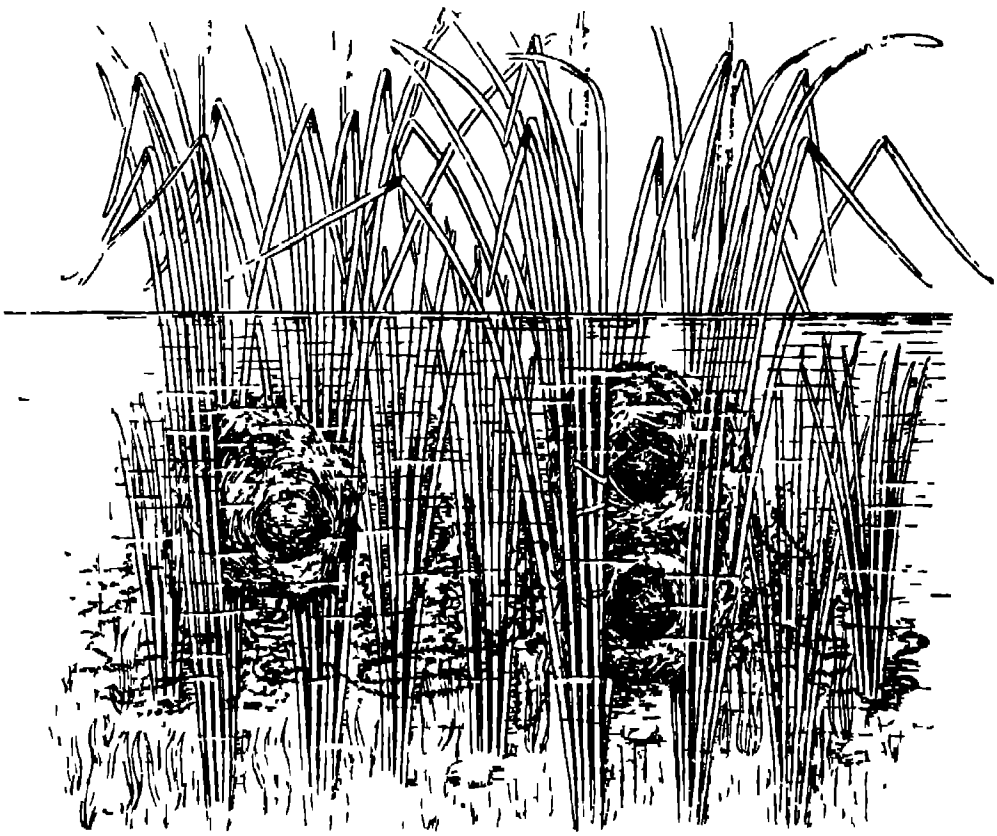

Fig 1 Nests of *Osphronemus goramt* (Lacépède) (Diagrammatic)

margin of the pond about 6 to 10 inches below the surface of water and about one foot above ground level. During our study three double nests (Fig. 1) one above the other have been noticed; in these it was observed that the upper nest was a discarded one without any eggs or larvæ in it and in a more disintegrated condition than the lower, the latter apparently constituting the second brood of the same pair of fish.

The nests were mostly made up of cut pieces of bulrush which grow profusely at the margin of the pond. Bulrush is a long gregarious robust

grass-like weed attaining a height of about 8 to 10 feet Pieces of bulrush are closely interwoven into a thick basket-like structure and to this groundwork are added aquatic grasses, twigs of other plants and sometimes even bits of rags that may be found at the margin of the pond The fact that plants like Elodea, Chara, Nitella, Vallisneria and others though found in plenty were assiduously kept out of the nest is interesting; probably the fish selects only plants which yield fibrous material for constructing the nest Further, the disintegrating softer tissue stimulates the growth of micro-organisms, particularly rotifers and infusorians within the nest, which are noticed abundantly among the fibrous tissue They form excellent food for the developing larvæ till they leave the nest Roxas and Umali (1937) have noticed nests being built among roots of water hyacinth in Philippines The fishes nibble leaves and stems with their hard jaws and soak the cut pieces in water for sometime, probably to render them more pliable and then carry them in their mouths for nidification. The fish takes about 8 to 10 days to build the nest.

While examining the nests it was noticed that whenever a nest was approached, a fish would scuttle away into the deeper portion of the pond. With a view to examine whether the fish guarding the nest was a male or a female, two separate nests were carefully encircled with a drag net on two occasions and the fish caught. On both the occasions the fish was a male with the characteristic hump on the forehead. It may therefore be concluded that it is the male that guards the nest In the case of another nest building Anabantoid, *Macropodus cupanus* (C and V), Jones states that the nest is always guarded by the male fish On another occasion a male fish was noticed nibbling grass in shallow water cutting it with a series of jerks.

Breeding

Roxas and Umali (1937) state that Gourami breeds throughout the year in Philippines. Kulkarni (1939) observes that the fish breeds in Bombay Presidency all the year round except during the monsoon from June to September According to Sundara Raj (1939), Gourami generally breeds twice a year in Madras—in February and March and again in September to November. The fish in our fish farm breeds practically throughout the year. Nests with eggs or larvæ have been noticed during August and October of 1943 and January, February, April, May, June and July of 1944. Active breeding has been noticed during summer months, April and May, when quite a large number of nests were constructed

We have not been successful in observing the actual process of spawning in spite of several efforts made in that direction. Several nests have been

noticed containing freshly-laid eggs. The eggs are deposited in the depressions in the nests and covered over with twigs and leaves with which the nest is constructed. The eggs are not adhesive and if they are not thus covered they float to the surface of water. The covering on the eggs not only keeps them within the nest but also protects them from being devoured by other fishes The mouth of the nest is just wide enough to admit the head of the fish inside. Probably the male contrives to transfer the eggs into the nest by its mouth and covers them. Otherwise it is difficult to explain how the floating eggs can be deposited and covered within the nest. Further attempts will be made to observe the mode of spawning and of deposition of the eggs in the nests. From one nest 1,450 just hatched larvæ were collected on 20th May 1943 Kulkarni (1939) has collected from the tank at Bandra two to three thousand larvæ from each nest.

Development

1. *Embryonic Development*

A study of the early development of Gourami was conducted during May 1944 The eggs were carefully taken out of a nest and brought to the laboratory attached to the Fish Farm and developed in glass trays. The embryonic and larval stages were examined under the microscope. The different stages of development have been fixed in 5% formaldehyde The pre-larval stages can be seen distinctly after the eggs are stained in Delafield hæmotoxylin and differentiated in acid alcohol.

The egg.—The eggs were collected at 10 A.M. on 13-5-44. They are not quite spherical but slightly oval in shape—2·7 mm. in diameter, the long axis being about 0·2 mm. longer. The size of the egg noticed by us is more or less in agreement with what has been stated by Kulkarni (1939) but Roxas and Umali (1937) record the diameter of the egg of Gourami as 1 mm. They are yellowish in colour and are semi-transparent. On account of the presence of clear fluid, which we are inclined to believe is fluid yolk (*f.yk.*) together with a few oil globules (*o.g.*) occupying about one-third of the egg at the abapical pole, the egg is actually inverted in the natural condition. The fluid yolk and the oil globules give buoyancy to the eggs. About two-thirds of the egg on the apical side constitutes a solid mass of yolk (*yk*). The vitelline membrane (*e.m.*) is thin and non-adhesive. By the time the eggs were collected from the nests the preliminary cell division was complete and a layer of blastoderm (*bl.*) had formed on the yolk mass of the apical pole (Fig. 3). The nest from which the eggs were taken was noticed to be empty on the previous evening and therefore it is presumed that this particular batch of eggs was laid during the early morning of the 13th May.

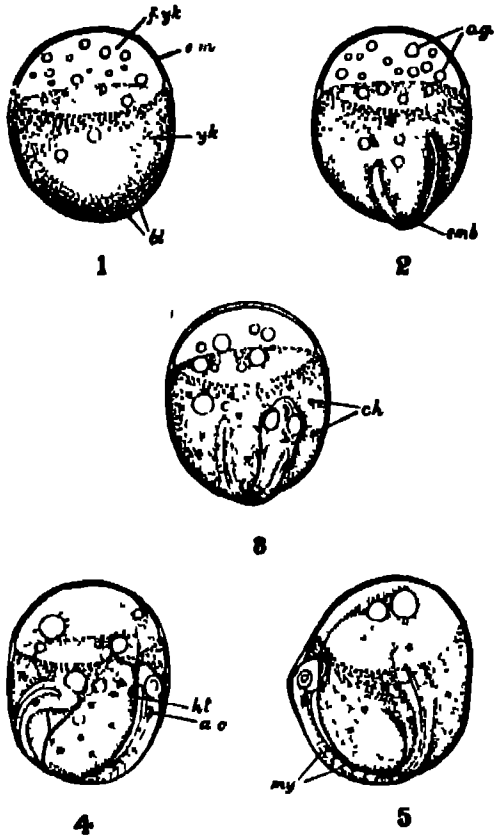

FIG. 3 *Osphronemus goramy* (Lacépède), Embryonic stages, approximately 1. 4 hours, 2. 6 hours, 3. 13 hours, 4. 27 hours, 5. 34 hours after fertilization of the egg × ca 8½

ac, auditory capsule, *bl*, blastoderm, *ch*, chromatophores, *em*, egg membrane, *emb*, embryo, *fyk*, fluid yolk; *ht*., heart, *my*, myotomes; *og*, oil globules, *yk*, yolk

By 12 noon the layer of blastoderm extends to the region of the fluid yolk at the abapical pole and faint indications of the formation of the embryo noticed. By 2 P.M. the blastoderm completely envelopes the yolk and the differentiation of the embryo begins. In the head could be seen broad divisions of the brain and the formations of the optic vesicles The caudal end is narrow. Two hours later 7 to 8 somites appear along the body. The fluid yolk becomes opaque on account of the extension of blastoderm over it. The embryo is still adpressed to the yolk mass. By 7 P.M. the number of myotomes increases to about 15 A few stellate cells appear on the yolk.

By 8 A M. next morning the embryo has grown considerably in size. The tail has developed a thin fin-fold and has become free from the yolk surface and lashes from side to side. The head is wider and thicker and

the eyes are proportionately large, but there is no pigment in them A little behind each eye could be seen the auditory capsule. The notochord and the nerve cord are seen distinctly. The divisions of the brain are well marked. About 20 to 25 myotomes can be counted (*my.*). The heart is just beginning to form beneath the head between the yolk and the outer layer of blastoderm. Gradually circulation is established The outline of blood vessels is not yet clear. By 4 P.M. there is active blood circulation and the different parts of the heart—sinus venousus, ventricle, auricle and the conus—can be distinguished The colourless blood flows forwards from the heart and coursing below the notochord proceeds to the end of the tail. Immediately below this is another vassel which conveys the blood forwards. About the middle of the body the vessel turns downwards conveying the blood to the yolk mass The blood collected from the yolk goes back to the heart by a thick vessel About this period some of the larvæ are seen hatching. It is seen that except for the tail the rest of the body is not free from yolk surface. The powerful lashing movement of the tail causes the rupture of the vitelline membrane and the larva comes out of the membrane. By about 6 P.M. all the larvæ have hatched out Hatchlings are seen darting out of

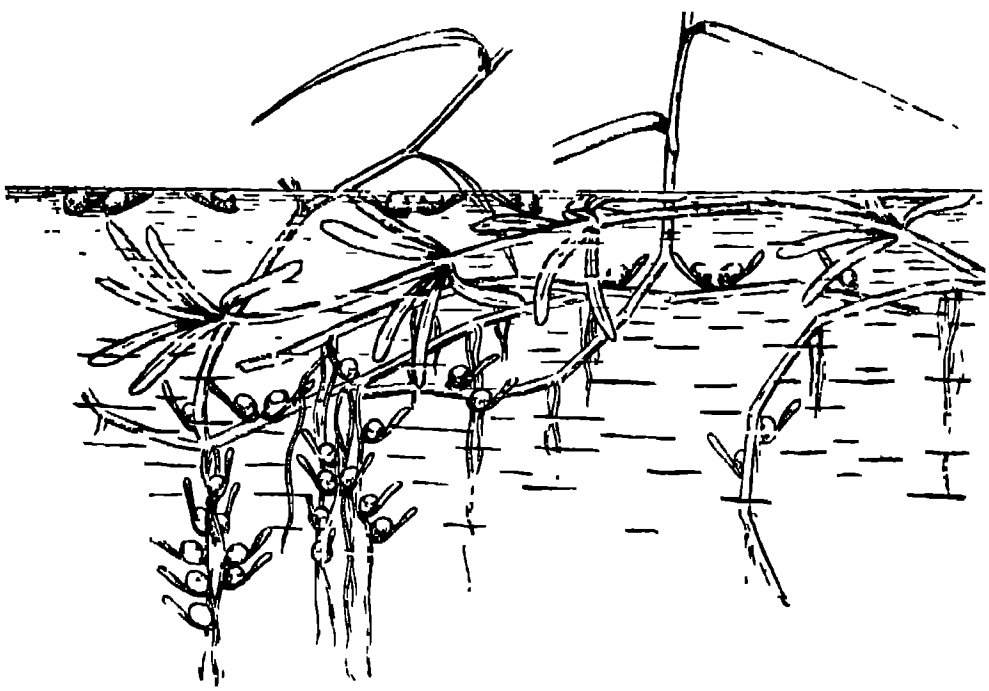

FIG. 2. The hatchlings of *Osphronemus gorami* (Lacépède) are shown attached to the aquatic plants and a few floating at the surface of water in a glass trough.

the egg membrane with a vigorous movement and float upside down at the surface of water on account of the presence of the fluid yolk and the oil globules at the abapical pole of the yolk sac and some of them attach themselves in groups to the roots and leaves of aquatic plants kept in the glass trough by the dorsal aspect of their heads as in Fig 2 A few are attached to the sides of the trough It is thus seen that the larvæ hatch out in about a day and a half. The period of the embryonic development of Gourami from the time of deposition of the eggs up to the hatching of larvæ varies in the same pond during different parts of the year It is short during summer and prolonged during winter. The larvæ hatched out in two and half to three days during August, in 5 to 6 days during January and February and in about a day and a half during May.

2. Larval Development

Newly hatched larva.—The newly hatched larva is in a remarkably poorly developed state The larva is transparent and measures 6 mm. in length and is closely pressed to the yolk mass except in the tail region. The mouth, gills, alimentary canal and the fins have not yet appeared. There is a thin median fin-fold extending along the dorsal and ventral aspects of the tail region The notochord is straight and unsegmented There is no pigment in the eye The blood is colourless About 25 myotomes are seen along the body. An interesting feature about the newly hatched larva is the presence of highly enlarged cells on the dorsal aspect of the head which exude a mucus secretion by which it attaches itself to the vegetation or any other object in water. An examination of the microscopic sections of the larva shows that these cells arise from the ectoderm and are much larger than the cells covering other parts of the body. It is also noticed that by the 8th day after hatching when the larva is actively moving about the secretory cells disintegrate giving place to normal ectodermal cells. The secretory cells on the head can be regarded as generalised adhesive or cement gland (Fig. 4, *c.gl*). The cement glands described by Jones in *Macropodus cupanus* appear to be more or less similar to those found in Gourami. In Teleosteans like *Hyperopisus bebe*, *Sarcodacus odæ* and *Etroplus maculatus* the secretory cells are localised giving rise to well-developed cement organs (Jones, 1937). A detailed account of the cement gland in Gourami will be published shortly. Though Kulkarni (1939) has noticed larvæ resting on the weeds at the bottom of the observation tank, he denies the presence of any cement glands in Gourami. During the first day of development there is a marked development in the vascularisation of the yolk mass and rapid absorption of yolk is noticed

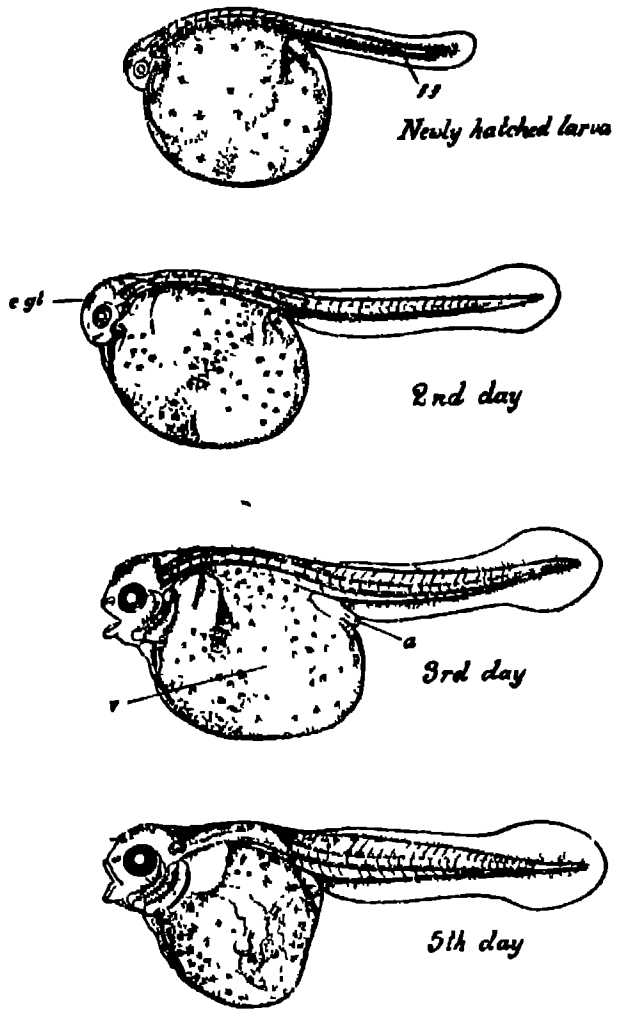

FIG 4 *Osphronemus goramí* (Lacépède), larval stages × ca. 8.
c.gl, cement gland ; *f f.*, fin-fold ; *v*, vacuity

2nd Day Larva.—The larva grows to about 8 to 9 mm. in length. The head becomes slightly lifted up from the yolk mass. Nasal capsules make their appearance. There is a gradual pigmentation of the eyes. The auditory capsules are drawn slightly nearer the eyes. The chondrocranium is forming. The mouth is just appearing as a slit but it is not yet functioning as it is covered up by membrane. The cells of the cement gland are prominently seen. The notochord is straight and unsegmented. Rudiments of the pectoral fins arise as small buds behind and below the auditory capsules The circulation of blood is becoming complicated. There are

vessels given out to the head and between myotomes. The number of chromatophores increase on the yolk sac and a few appear on the body.

3rd Day Larva.—The larvæ though attached are more active. The head and the body have become thick. The eyes are large with black pigment. The mouth has appeared but the jaw bones are not yet developed. The gill arches and rudimentary gill filaments are formed. The larva has commenced to breathe. The blood has become red and can be seen flowing into the gill filaments. The heart is functioning at a rapid rate of about 180 beats a minute. The pectoral fins have grown longer and vibrate rapidly. The anus appears as an invagination at the angle between the yolk sac and the body. The alimentary canal is not yet seen Considerable yolk has been absorbed by now and in its place a clear vacuity appears which gradually increases in size.

4th and 5th Day Larva.—This period is marked by the larvæ setting themselves right in position. The larvæ which were floating or attached to aquatic plants hitherto in an inverted condition gradually assume correct position and move about freely. This is aided, as could be seen from the specimens, by the upward extension of the yolk sac with the vacuity as a pouch-like structure, one on either side of the body (Fig 5, *lp*). In a microscopic section of the trunk region of the larva at this stage, the pouches can be seen distinctly. In the head the chondrorcranium, the jaw bones and the opercular bones are formed. The opercular opening is distinctly seen and the larvæ respire rapidly. The anterior portion of the alimentary canal is not clearly seen as it is covered by the yolk. The intestine, the wall of which consists of large cells, forms a loop and opens by the anus; the liver forms as sac-like evagination from the gut and is green in colour. A small pre-anal fin-fold is seen. By the end of the 5th day a slight upward bend of the hinder end of the notochord is observed. The heart is pushed up and the yolk circulation has become feeble. The post-cardinals have formed.

6th and 7th Day Larva.—Except for the thin mass of yolk along the margin of the yolk sac the yolk has been fully absorbed. The yolk sac is reduced in size and pushed upwards. It would be necessary to make it clear that the yolk sac is no longer a separate unit. It is already stated that the blastoderm covers the yolk sac completely with the result the yolk sac has to be regarded as having been taken inside the body. The notochord becomes segmented and the upward extension of the hinder end is more pronounced giving the caudal region a heterocercal condition. The rudiments of the caudal rays are formed. The neural and hæmal arches can

also be seen. The jugular veins and the hepatic portal vein are formed during this period. The larvæ begin to feed on the micro-organisms. They were fed in the laboratory with finely crushed boiled fowl's egg which they devoured with avidity

8th and 9th Day Larva.—With the increasing development of musculature and bones, especially in the head, the larva is becoming thick and opaque. The larva is gradually assuming fish form by further diminution of the yolk sac region The chromatophores become numerous and prominent all over the body The air bladder is seen developing as an evagination from the dorsal aspect of the anterior part of the gut (Fig. 5, *a b.*) The

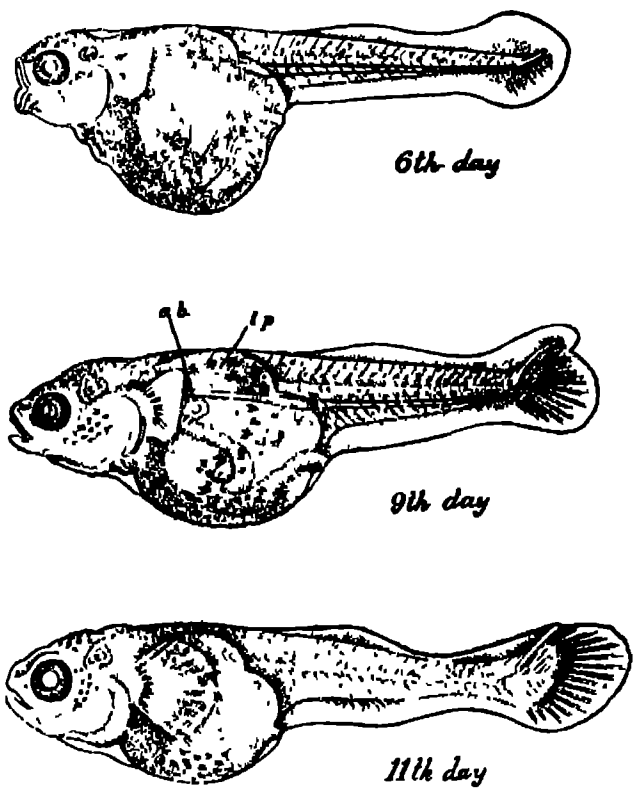

FIG. 5. *Osphronemus goramí* (Lacépède), larval stages × ca. 8.
a b, air bladder ; *l.p*, lateral pouch of the yolk sac region.

median fin-fold is gradually diminishing in size. The caudal fin is well defined assuming a homocercal condition and the caudal rays are formed.

10th to 15th Day Larva.—The larvæ are gradually growing in bulk. The yolk sac region now presents the normal contour of the body. The

heart has become thick and occupies the normal position in the thorax. By this period practically the full compliment of the circulatory system of the adult fish has developed. The dorsal and the ventral fins are now distinct from the caudal and have developed rays. It is noticed that the larvæ leave the nest between 15th and 18th day after hatching.

1 *Month Old Larva.*—The whole body is covered with very large number of chromatophores. Nine broad vertical stripes are noticed. The head has become considerably big with large eyes. The scales and the ventral fins have not yet developed.

On 12th May 1944 a shoal of young Gourami was noticed in the pond. A few of them were netted and found to measure 18 mm. in length. The scales and ventral fins were developed but the long ray of the ventral fin was still lacking. The spinous rays of the dorsal and anal fins were present. There was a big dark blotch on the soft rays of the dorsal fin. The young fish had the exact shape and form of the adult. These are probably the offspring of breeding during the end of January or the beginning of February.

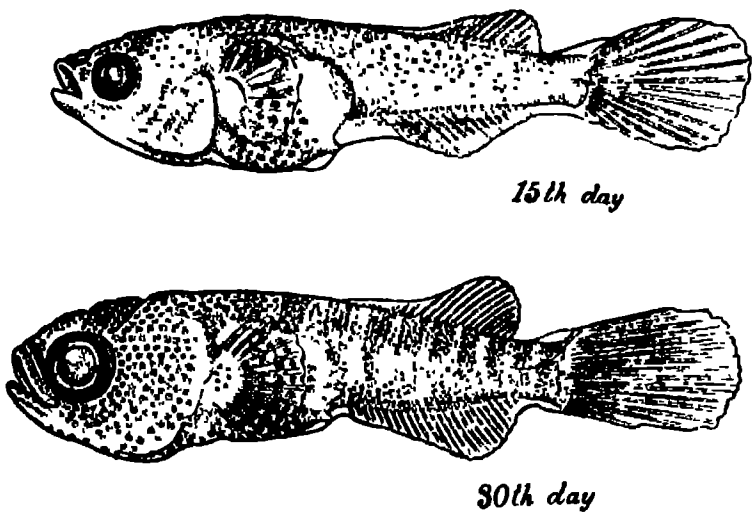

FIG. 6. *Osphronemus gorami* (Lacépède), larval stages. × ca 8

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Summary

Experiments on the acclimatisation of Gourami at the departmental fish farm at Markonahalli (2,400 feet above sea-level) in Mysore State have proved successful. The fish are thriving well and are breeding freely.

Gourami builds a nest of aquatic plants for the reception of its eggs. The nest is more or less ovoid in shape with a shallow depression. It is built among the vegetation along the margin of the pond and is attached to the long blades and stems of bulrush, a little above the ground level and a few inches below the water surface It takes about ten days for the fish to construct the nest. Generally it is the male fish that guards the nest after spawning

Gourami appears to breed practically throughout the year The eggs are buoyant due to the presence of fluid yolk and oil globules in them. They are deposited in the nest and covered over with leaves and twigs. The embryonic development is rapid and the egg hatches in about 36 hours The newly hatched larva is highly immature without the mouth, gills and fins. The larvæ either float upside down or are attached to the aquatic plants by means of cement glands present on the dorsal aspect of the head. At the time of hatching the blood circulation has started and the yolk is being absorbed. Rudiments of pectoral fins appear on the second day after hatching. The mouth, anus and gills appear on the third day when the larva commences to breathe. By the fourth or the fifth day the larva sets itself right and the alimentary canal is seen in the vacuity caused by the absorption of the yolk. The larvæ commence feeding on the micro-organisms from the fifth day. By the eighth day the larva becomes opaque with the increasing development of muscles and bones. Gradually the dorsal, caudal and anal fins become differentiated. Within a period of fifteen days the full compliment of the circulatory system of the adult fish is developed. During the third week the larvæ leave the nest The ventral fins do not appear even in a month old larva. Young Gourami of about four months old, measuring 18 mm. in length, have the form and shape of the adult and have developed scales, ventral fins and spiny rays of the dorsal and anal fins.

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STUDIES ON THE COCCIDIA OF INDIAN BIRDS

II. Observations on Several Species of Coccidia of the Sub-Families Cyclosporinæ and Eimeriinæ.

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CONTENTS

Introduction

This paper is the continuation of the series in our studies on the coccidia from Indian birds and embodies the results of our observations on several species of coccidia of the sub-families Cyclosporinæ and Eimeriinæ. The parasitic species described here are new to science, except *Eimeria pfeifferi* (Labbé, 1896) which is reported here for the first time from this country.

The birds were either collected from the suburbs of Calcutta or purchased from local dealers. Ten different species of birds were examined for their protozoan parasites and it happened that most of them harboured the parasites. The table at the end of this paper shows the occurrence of the parasites in different species of birds and the number of birds found infected out of the total number examined. The seat of infection and the locality of the parasites and the hosts are also noted in the table.

For the maturation of the oöcysts 1 per cent. chromic acid solution or 2·5 per cent. potassium bichromate solution was used. For the study of the endogenous stages, the intestine of the infected birds was fixed in Bouin-Duboscq and Brasil's fluid for 24 hours. 6-12 μ thick sections were cut; they were stained with Delafields or iron-alum hæmatoxylin.

We have great pleasure in recording here our thanks to Dr. S. C. Law, the eminent ornithologist of Calcutta, for his identification of the birds used here.

Sub-Family Cyclosporinæ. Wenyon (1926)

This sub-family* includes three genera, *viz.*, *Cyclospora*, *Isospora* and *Dorisiella* The species recorded here belong to the last two genera

Genus Isospora *Schneider* (1875)

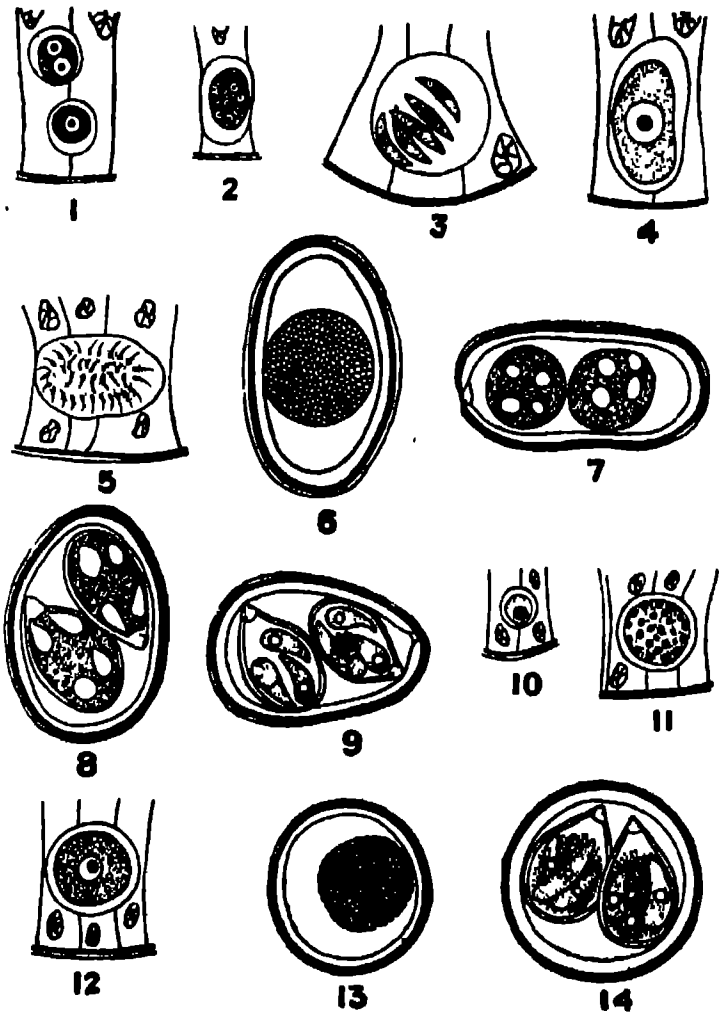
The only species of *Isospora* so far known from Indian birds is *I. lacazei* (Labbé) reported by the writers (1944) During the course of our investigation, we found the following birds namely the black-headed munia *Munia malacca malacca* Linn., the bank myna *Acridotheres ginginianus* (Lath.), and the black-headed myna *Temenuchus pagodarum* (Gmel.) infected with three distinct species of *Isospora*; as these parasites do not resemble any known species of *Isospora* we propose to call them *Isospora muniæ* n.sp. *Isospora ginginiana* n.sp. and *Isospora temenuchii* n.sp. respectively after the names of their hosts. The black-headed munia was found to be infected with a species of *Dorisiella* and *Eimeria* also.

Observations on Isospora muniæ *n.sp.*

Schizogony.—The youngest trophozoites (Fig. 1) encountered in the sections of intestine of the host are spherical in outline and measure 4·4 μ in diameter They have nucleus which is placed centrally and consists of a deeply stained chromatic granule with a clear space around it.

* The classification of the suborder Eimeriidea as given by Hoare (1933) is followed in this work.

The schizonts (Fig. 2) are elongated, oval in shape; the full grown forms measure 10·0–13·2 $\mu \times 6 \cdot 6$–8·8 μ. In a mature form sixteen nuclei can be seen. The nuclei have the same characteristic feature as in the previous stage.



Figs 1–9. *Isospora munia* n sp —Fig 1 An uninucleate trophozoite and a binucleate schizont. Fig. 2 A schizont showing nuclei Fig. 3 A group of merozoites Fig. 4 A macrogametocyte Fig. 5 A microgametocyte showing fully developed microgametes. Fig. 6 An unsegmented oöcyst Fig 7. An oöcyst showing spherical sporoblasts Figs. 8 and 9 Mature oöcysts. Figs. 10–14. *Isospora ginginiana* n sp.—Fig. 10 An early trophozoite Fig. 11. A microgametocyte. Fig. 12. A macrogamete. Fig. 13. An unsegmented oöcyst. Fig. 14 A mature oöcyst. (All figures are magnified 1066 times.)

The merozoites (Fig. 3) are crescentic in appearance and measure 8·8 $\mu \times 1 \cdot 5 \mu$. They have a uniformly granular cytoplasm. The nucleus

is placed at the centre of the body and is provided with a small deeply stained chromatin mass which is contained within the nuclear membrane.

The endogenous cycle of development of the parasite takes place in the small intestine Stages representing schizogony are found in the gland cells of the sub-mucous membrane while those of sporogony occur in the mucous membrane.

Sporogony.—The young macrogametocytes are spherical in shape and measure 8·8 μ in diameter The cytoplasm is highly granular and contains a circular nucleus The latter has a large karyosome and a nuclear membrane. As the macrogametocytes increase in size, they become oval in shape (Fig. 4) and their cytoplasm becomes vacuolated. The mature forms measure 13·2 μ × 18·8 μ. The macrogametes have the same form and size as the mature macrogametocytes, but the cytoplasm of the former presents deep staining inclusions which are regarded by many as reserve food-material. These inclusions usually occupy the peripheral region of the macrogametes. The nucleus of the macrogamete measures about 4·5 μ in diameter

The microgametocytes are also oval in shape and contains several small vesicular nuclei arranged along the periphery The fully grown microgametocytes measure 11·0 μ × 15·4 μ The microgametes are small comma-shaped bodies (Fig. 5) with both the extremities pointed. Each microgamete measures 4 4 μ in length and is provided with a long flagellum at one end.

Both the mature and the immature oöcysts are broadly or elongately oval. They are provided with a micropyle. The unsegmented zygote contained within the immature oöcyst is spherical in shape measuring about 14·5 μ in diameter. Its cytoplasm is highly granular and contains several refringent globules (Fig. 6) The oöcysts possess a double-layered envelope of about 1 0-2·0 μ thick and they measure 24·7–30 9 μ × 14·4–18·5 μ. Sporulation takes place after 48 hours when two circular sporoblasts appear without leaving any oöcystal residuum (Fig. 7).

The sporocysts when fully formed are pyriform in shape with the anterior end pointed and the posterior rounded (Figs 8 and 9). The sporocysts are also double-layered and each of them is provided with a refractile knob at the pointed anterior end. After the development of the sporozoites, the sporocystic residue is seen scattered irregularly within the sporocyst. The sporocysts measure 14·4–16·5 μ × 10·3 μ.

The sporozoites measuring 6·6 μ × 2·2 μ are sickle-shaped bodies arranged irregularly within the sporocyst (Fig 9). Their anterior end is pointed and the posterior end rounded where the nucleus is situated.

Affinities.—Of the known species of *Isospora* from birds the parasite under report has some resemblances with *I. volki* Baughton (1937). The oöcysts of *I. muniæ* approach those of *I. volki* in shape but differ in size. Absence of micropyle, shape of the sporocysts and the structure of the sporozoites in *I. volki* at once distinguish it from this new coccidian.

Diagnosis—

Systematic position—*Isospora muniæ* n.sp. (Coccidiida, Eimeriidæ).

Description.—Oöcysts broadly oval or elongately oval, 24·7–30·9 μ × 14·4–18·5 μ, with a micropyle, no oöcystic residuum; sporocysts pyriform, with a knob at the pointed end, 14·4–16·5 μ × 10·3 μ, sporocystic residual mass present; sporozoites sickle-shaped, irregularly arranged, 6·6 μ × 2·2 μ

Seat of Infection.—Small intestine.

Host.—*Munia malacca malacca* Linn.

Locality—Calcutta.

Observations on Isospora ginginiana *n.sp*

Schizogony.—Only a few stages representing schizogony were found in our preparations. The stages that we could observe are the earliest trophozoites and the schizonts; the former are circular in outline, possess a chromatin granule which represents the nucleus (Fig. 10) and they measure 2·2 μ in diameter The schizonts measuring 8·8 μ in diameter are also circular in outline and have 8–16 nuclei scattered irregularly in the cytoplasm.

Sporogony.—The microgametocytes (Fig. 11) measuring 11·0–13·2 μ in diameter, are perfectly spherical in shape and they have an uniformly granular cytoplasm. The nuclei consisting of minute chromatin dots are arranged along the periphery. The mature microgametes are comma-shaped in structure and each of them is provided with a short flagellum at one end.

The macrogametocytes are also spherical in shape and have highly granular cytoplasm containing a nucleus. The nucleus has a membrane around it and an excentrically placed karyosome. The mature macrogametes (Fig. 12), 11·0 μ in diameter, have the same structure as the macrogametocytes, but the cytoplasm of the former is provided with food-material which occurs in isolated heaps and takes up deep stain.

The unsegmented oöcysts (Fig. 13) were seen to pass out with fæces of the host. The oöcysts, both mature and immature, are perfectly rounded in shape measuring 22·0–24·2 μ in diameter. The unsegmented zygote within the immature oöcyst is always spherical in shape and measures 12·0–15·4 μ in diameter. Neither micropyle nor oöcystic residuum is present.

The sporocysts, when fully grown, are typically pyriform in shape and have pointed anterior and rounded posterior extremities (Fig. 14). The pointed end is provided with a refractile knob. The sporocysts measure 15·4–17·6 μ × 11·0 μ.

The sporozoites are elongated in shape, with their anterior end pointed and the posterior round. They measure 11 μ × 4 μ. The cytoplasm is clear and a spherical nucleus is situated at the centre.

Affinities.—Isospora lacazei (Labbé, 1893) and *Isospora lyruri* Galli-Valerio (1931) resemble the coccidium under report in the shape of oöcyst, all of them being spherical in shape. In size the oöcysts of *I. lyruri* are much smaller than those of *I. ginginiana*. The latter species differs from *I. lacazei* in the structure of the sporocysts and the sporozoites.

Diagnosis—

Systematic position.—Isospora ginginiana n.sp. (Coccidiida, Eimeriidæ).

Description.—Oöcysts rounded, 22·0–24·2 μ in diameter, no micropyle and oöcystic residuum; sporocysts pyriform, with a knob at the pointed end, 15·4–17·6 μ × 11·0 μ., sporocystic residuum present, sporozoites elongated and irregularly arranged.

Seat of infection.—Intestine.

Host.—Acridotheres ginginianus (Lath.).

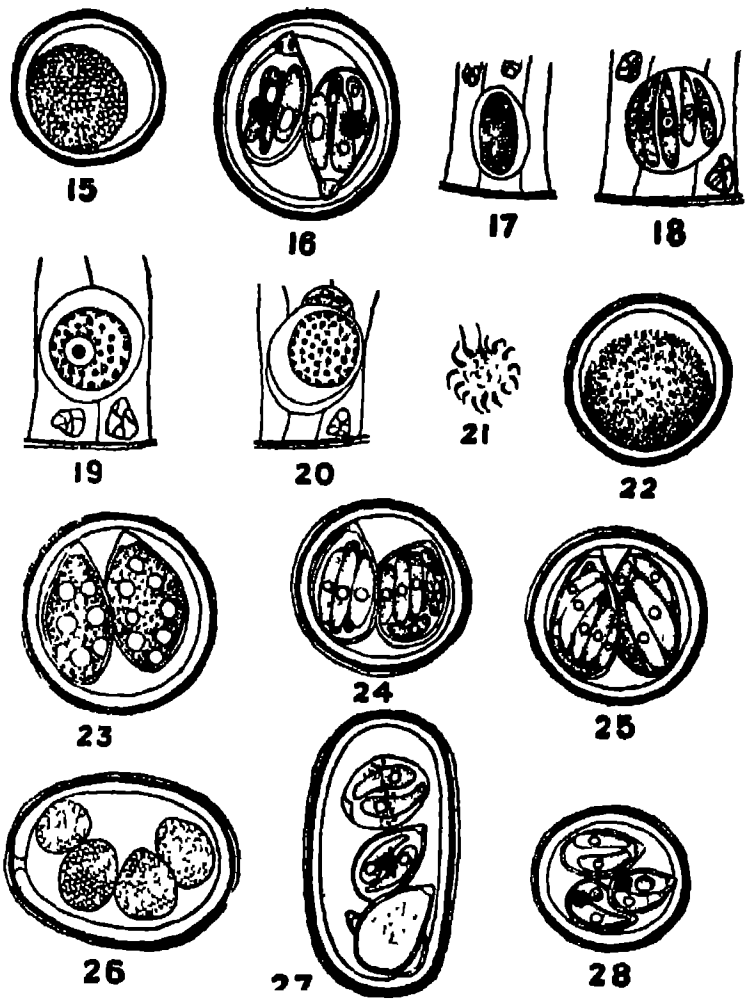
Locality.—Calcutta

Observations on Isospora temenuchii *n sp.*

The endogenous stages of this coccidian could not be found by us; probably the host bird was examined by us at the late stage of infection. Only immature oöcysts were found to come out in large numbers with the fæcal matter of the bird.

The majority of the oöcysts are subspherical in shape while a few are slightly ovoid and they measure 22 0–24·2 μ × 19·8–22·0 μ. A micropyle could be detected in a few of the oöcysts. The oöcystic wall is about 2 μ thick and double layered. The unsegmented zygote (Fig. 15) is highly granular and contains several refringent globules. It measures about 15·5 μ in diameter. The zygote divides equally to give rise to the two sporoblasts leaving no oöcystal residue.

The sporoblasts first appear as spherical bodies but soon they become elongated. The fully developed sporocysts are elongately oval in shape (Fig. 16), with the anterior end somewhat bluntly pointed. At this narrow



Figs. 15 and 16 *Isospora temenuchii* n.sp.—Fig. 15. An unsegmented oöcyst. Fig 16 A mature oöcyst. Figs. 17–25 *Doristella harent* n.sp.—Fig. 17 A schizont Fig. 18 A group of merozoites Fig 19 A macrogamete Fig 20 A microgametocyte. Fig. 21. A group of microgametes From a smear. Fig. 22 An unsegmented oöcyst. Figs. 23–25 Oöcysts in different stages of development Figs 26 and 27 *Eimeria malaccæ* n.sp —Fig. 26. An oöcyst showing the differentiation of sporoblasts Fig 27. A mature oöcyst. Fig 28 *Eimeria pfeifferi* (Labbé)—A mature oöcyst. (All figures are magnified 1066 times.)

end there is a well-developed knob. The sporocysts measure 15·4–17·6 μ × 11 0 μ, the maximum width being at the middle region of the sporocyst. The sporocystal residual body is well marked after the differentiation of the sporozoites and accumulates in the form of a bunch which is about 5·0 μ in diameter.

The sporozoites are elongated in shape with the anterior end narrowing down to a blunt point while the posterior end remains round. The nucleus which is spherical or slightly oval, is placed near the centre of the body or slightly towards the rounded end of the sporozoite The sporozoites measure $8{\cdot}8\mu \times 3\ 3\mu$.

Affinities.—This parasite resembles *Isospora nucifragæ* Galli-Valerio (1933) and *Isospora strigis* Yakimoff and Matschaulsky (1936) but differs from both of them in many respects *I. temenuchii* differs from *I. strigis* in having a micropyle, a well-marked sporocystic residuum and in the structure of the sporozoites

Diagnosis—

Systematic position.—Isospora temenuchii n sp. (Coccidiida, Eimeriidæ).

Description.—Oocysts sub-spherical or slightly oval, $22{\cdot}0$–$24\ 2\mu \times 19\ 8$–$22{\cdot}0\mu$ micropyle present, no oocystic residuum; sporocysts elongately oval, with a knob at the pointed end. $15{\cdot}4$–$17\ 6\mu \times 11{\cdot}0\mu$, sporocystic residuum well marked; sporozoites elongated.

Seat of infection —Intestine

Host —Temenuchus pagodarum (Gmel.).

Locality —Calcutta

Genus Dorisiella *Ray* (1930)

The genus *Dorisiella* was established in 1930, by Ray to accommodate the species *scolelepidis* found in the alimentary canal of the polychæte worm *Scolelepis fuliginosa* Claparede, occurring at Plymouth. Since then another species *D. hoarei* has been described by Yakimoff et Gousseff (1935) from the serpent *Elaps quatuor-lineata sauromates* Pallas. The species found by us in the alimentary canal of the bird *Munia malacca malacca* Linn. is new to science and is the third representative of the genus. For the first time it is recorded from an avian host, and is the only member of the genus so far reported from India. We propose to call it *Dorisiella hareni* n.sp after the founder of the genus.

Observations on Dorisiella hareni *n.sp.*

Schizogony.—The stages representing schizogony occur in the gland cells of the submucosa and they are restricted in the small intestine of the host.

The youngest trophozoite, about 4μ in diameter is circular in outline. The cytoplasm is very clear and it contains a nucleus which consists of a

chromatin mass of very small size. The binucleate schizonts have the same characteristic features as shown by the uninucleate trophozoites.

The full grown schizonts (Fig. 17). measuring $13 \cdot 2\mu \times 6 \cdot 6\mu$ are oval in shape and possess sixteen chromatin granules which are regarded as the precursors of the nuclei of the merozoites. The cytoplasm of these stages are uniformly granular.

The merozoites (Fig. 18) are elongated in form having one of the ends pointed and the other rounded. The cytoplasm of the merozoites is uniformly granular. The nucleus, at the centre, is circular in outline and consists of a small chromatin granule surrounded by a delicate membrane. They measure $8 \cdot 8\mu \times 2\ 2\mu$.

Sporogony.—Both the macro and the microgametocytes were found in large numbers in the mucous-membrane of the small intestine.

An early macrogametocyte is spherical in shape and its nucleus contains a karyosome which is comparatively larger than the chromatin mass of the early trophozoite; the nucleus possesses a well-defined nuclear membrane. The cytoplasm of these forms is uniformly granular. As the macrogametocytes grow in size the cytoplasm becomes vacuolar and in a still later stage heaps of food material are seen to accumulate within the vacuoles. These latter stages represent the mature macrogametes (Fig 19) which retain the shape of the early macrogametocytes and measure $13 \cdot 2\mu$ in diameter.

The fully grown microgametocytes (Fig. 20), measuring 9 8μ in diameter, are also spherical in outline and contain a large number of dot-like chromatin granules arranged along the periphery. The chromatin granules become vesicular and give rise to the microgametes (Fig 21) The latter are curved bodies having both the extremities pointed. From one of the ends of a microgamete a short flagellum is developed.

The fertilized macrogametes can be distinguished from other stages by the nature of the cytoplasm, which on surface view looks like a powdery mass and also by the structure of the nucleus. The nuclear membrane is ill-defined and the karyosome stains faintly.

The unsegmented oöcysts (Fig. 22) were discharged in large numbers with the fœces of the host. Both the immature and mature oöcysts are spherical in contour and possess a double envelope which is about $2 \cdot 0\mu$ thick. No micropyle could be seen at any stage of development of the oöcysts. The oöcysts measure $18 \cdot 5\mu$–$22 \cdot 6\mu$ in diameter with an average of $20 \cdot 6\mu$. The unsegmented zygote, which is circular in outline, is highly granular and contains several refringent globules. The zygote divides into

two sporoblasts leaving no residuum in the oöcyst and the approximate time required for its division in 2·5 per cent. potassium bichromate solution, is about 24 hours.

The sporoblasts when they first appear, are ovoidal but soon they assume typical pyriform shape (Figs. 23–25). At the anterior pointed end of the sporocyst there is a knob which is not well developed.

The sporocysts measure 14·4–18·5 μ × 9·3–10·3 μ They reach full maturity in two days when sporozoites begin to differentiate out in the form of spherical bodies (Fig. 23). The sporozoites (Figs. 24–25) are fully-formed after 3 or 4 days leaving a diffuse sporocystic residual mass

The sporozoites are club-shaped and have bluntly pointed anterior end. The nucleus is situated near the posterior rounded end of the sporozoite which measures 8·2 μ × 2·2 μ.

Affinities.—The parasite under report differs from the type species *D. scolelepidis* in the shape of the oöcyst* and in the structure of the sporocysts which are oval in shape in the latter Moreover, there is a considerable difference between the endogenous stages of *D. hareni* and *D. scolelepidis*.

Dorisiella hoarei Yakimoff et Gousseff (1935) resembles the present coccidium both in the shape and size of the oöcysts. In spite of this resemblance, they differ in the structure of the sporocysts and the sporozoites, which are of diagonistic value.

Diagnosis—

Systematic position.—Dorisiella hareni n sp. (Coccidiida, Eimeriidæ).

Description.—Oöcysts spherical, range 18·5–22·6 μ with an average 20·6 μ, micropyle and oöcystic residuum not present, sporocysts pyriform with a knob at the pointed end, range 14·2–18·5 μ × 9 3–10·3 μ, sporocystic residuum diffuse; sporozoites club-shaped measuring 8·2 μ × 2·2 μ.

Seat of infection.—Intestine.

Host.—Munia malacca malacca Linn.

Locality.—Calcutta.

SUB-FAMILY EIMERIINÆ WENYON (1926)

Of the three genera of this sub-family only one genus namely *Eimeria* is represented here.

* Hoare (1933) has pointed out that Ray (1930) in fig. 17, pl XLI in his paper has shown a line connecting the two sporoblasts, though Ray stated that there is no common envelope or oöcyst in *D. scolelepidis*. The line connecting the sporoblasts is regarded by Hoare as the oöcyst membrane.

Genus Eimeria *Schneider* (1875)

The only species of this genus described from the avian fauna of this country is *Eimeria columbæ* Das-Gupta (1938) and we add here two more species to the list. The first is a new coccidian which we propose to call *Eimeria malaccæ* n.sp., found in the alimentary canal of the bird *Munia malacca malacca* Linn. and the second is the well-known species *Eimeria pfeifferi* (Labbé) obtained from the Indian pigeon *Columba livia intermedia*, Strickl.

Observations on Eimeria malaccæ *n.sp.*

Only a few oöcysts of this coccidium were found by us, the endogenous stages could not be found in our preparations.

The oöcysts both mature and immature, are either broadly oval or elongatedly oval in structure and measure 26·8–30·9 μ × 16·4–18 5 μ. A micropyle is present in the oöcyst but no oöcystic residuum is found after the formation of the sporoblasts (Fig. 26).

The sporocysts (Fig. 27) could be seen to develop in only one oöcyst and the shape of the former is more or less oval with the anterior end pointed. A small knob can be seen at the anterior pointed end of the sporocyst which measures 12·4 μ × 10·3 μ. A diffuse sporocystic residuum is present. The sporozoites, measuring 8·3 μ × 2·1 μ, are elongated in shape with the anterior end tapering. The nucleus is placed near the posterior end of the sporozoite.

Affinities.—The oöcysts of the parasite under report approach those of *Eimeria caucasia* Yakimoff and Buewitsch (1932), *E. dispersa* Tyzzer (1929), and *E. johnsoni* Yakimoff and Rastegaieff (1931), *E. melangridis* Tyzzer (1929), and *E. tyzzeri* Yakimoff and Rastegaieff (1931) in size, but differ from them in other characters.

Diagnosis—

Systematic position.—Eimeria malaccæ n.sp. (Coccidiida, Eimeriidæ).

Description.—Oöcysts broadly oval or elongately oval, range 26·8–30·9 μ × 16·5–18·5 μ, micropyle present but no oöcystic residuum; sporocysts oval with anterior end pointed bearing a small knob, 12·4 μ × 10·3 μ, diffuse sporocystic residuum present; sporozoites elongated with anterior end tapering and measure 8·3 μ × 2·1 μ.

Seat of infection.—Intestine.

Host.—Munia malacca malacca Linn.

Locality.—Calcutta.

Observations on Eimeria pfeifferi (*Labbé*)

This coccidian has been studied by various workers from different parts of the world. It is reported for the first time from this country.

The oöcysts (Fig. 28) were obtained by us from the fæcal matter of a number of pigeons. They are either oval or spherical in shape, the oval form measures 19·8–20·9 μ × 16·5–17·6 μ while the spherical one is 17·6–18·7 μ in diameter. The range in size of the oöcysts as given by Wenyon (1926) varies 15·0–26·0 μ × 14·0–24·0 μ. Wenyon states "There is no evident micropyle in the oöcyst, and no residual body is formed within it, but a large one appears in each of the sporocyst." The latter part of his statement needs no comment, but it should be noted here that a small micropyle occurs in the majority of the oöcysts examined by us. Nieschulz (1925) has delineated a micropyle-like structure in Figs. 11 and 14 of his Pl. 17, but no mention of it has been made in the text

The sporocysts have the same characteristic features as described by previous workers and they measure 11·0–13·2 μ × 6·6 μ. The sporocystic residuum is accumulated into a mass and appears light green in colour under reflected light.

The sporozoites which are elongated bodies, also appear light green in colour and measure 6·6 μ × 2·2 μ.

TABLE

Host	Number examined	Number infected	Parasite	Locality
Acridotheres ginginianus (Lath.)	2	1	*Isospora ginginiana* n.sp	Calcutta
Acridotheres tristis tristis (Linn)	3			,,
Ardeola grayii (Sykes)	6			,,
Columba livia intermedia Strickl.	12	10	*Eimeria pfeifferi* (Labbé)	,,
Eudynamis scolopaceous scolopaceous (Linn.)	3			,,
Halcyon smyrnensis smyrnensis (Linn.)	1	.	.	,,
Munia malacca malacca Linn. ..	6	6*	*Isospora munia* n sp.	,,
			Doriella hareni n.sp.	,,
			Eimeria malacce n.sp	,,
Oriolus xanthornus xanthornus (Linn)	1	..		,,
Psittacula cyanocephala cyanocephala (Linn.)	7	.		,,
Temenuchus pagodarum (Gmel.)	2	2	*Isospora temenuchii* n sp.	,,

* All the birds were infected with *Doriella hareni*, four with *Isospora munia* and one with *Eimeria malacce*.

Summary

1. Three new species of *Isospora* have been described.

2. A new species of the genus *Dorisiella* is recorded and described for the first time from an avian host and also from India.

3. A new species of *Eimeria* is described.

4. *Eimeria pfeifferi* (Labbé) is reported for the first time from this country.

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* Not available to the authors.

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ERRATA

Vol. XX, No. 4, Sec B, October 1944

Page 115, line 4 (title) Delete commas before and after "eggs"
Page 115, line 23 Give paragraph spacing to line beginning with "Two species".
Page 118, line 2. Insert fullstop after "exposed".
Page 119, line 21. Insert comma after "barnacles".
Page 119, line 25. For "eggs" read "legs"
Page 120, line 21 Insert full-stop and dash after "4".
Page 120, line 26. Insert dash after "C"
Page 124, line 13. For "*Cuticula*" read "*cuticula*"
Page 126, line 7. For "in crease" read "increase".
Page 129, line 18. Delete comma after "(7)".

SOME OBSERVATIONS ON THE BREEDING BIOLOGY, AND ON THE SWELLING, WEIGHT, WATER-CONTENT AND EMBRYONIC MOVEMENTS IN THE DEVELOPING EGGS, OF THE MOLUCCAN KING-CRAB, *TACHYPLEUS GIGAS* (MÜLLER) [ARTHROPODA, XIPHOSURA]

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(With 1 Plate and 2 Text-Figs.)

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CONTENTS

1. Introduction

Two species of king-crabs occur on the east coast of India. These are *Tachypleus gigas* (Müller) [= *Limulus moluccanus* Latreille] and *Carcinoscorpius rotundicauda* (Latreille) [= *Limulus rotundicauda* Latreille]—*vide* Pocock, 1902. Of their development and biology in India we know so little that a few observations that I was able to make some time ago on the eggs of *T. gigas* are worth recording. The only previous account of *T. gigas* is in the excellent work of Iwanoff (1907, 1923, 1924, 1933) who collected its eggs in Java in 1906. He did not study egg-weights, embryonic movements, etc., which I have recorded, and in some points regarding egg-swelling my observations differ from his.

Acknowledgments.—It is a pleasure to tender acknowledgments for help to the following:—To Drs. S. L. Hora and B. N. Chopra for generously

handing over to me the entire egg-material that they collected, the latter also supplying valuable field data from memory; to Dr. B. Prashad, the Director, for kindly identifying the specimens of king-crabs; to Dr. R. K. Dutta-Roy, Chemist, Geological Survey of India, for valuable assistance in placing his laboratory resources at my disposal for the weighing and drying of eggs; to the Meteorologist, Alipore Observatory, Calcutta, for supplying meteorological data; and finally, to Mr. H. K. Bose, Assistant in the Zoological Survey of India, for help in the calculations.

2 *Material and Technique*

Several hundreds of eggs collected by Drs. S. L. Hora and B. N Chopra, on March 8 and 9, 1941, near Chandipore beach (Balasore District, Orissa) and brought to Calcutta in the wet mud in which they were found, were reared by me in ordinary rooms in the Indian Museum The mud was moistened from time to time with sea-water brought from the place of collection. The eggs throve excellently for about 3½ weeks (up to about April 4) when, for some reason, the embryos became sluggish and died The eggs throve equally well between pads of cotton moistened with sea-water. Iwanoff (1933) too could not rear them beyond 3 weeks in Java. During the rearing period (March 10–April 4, 1941), the daily shade temperatures (in °F.) of Calcutta, as supplied by the Meteorologist, Alipore Observatory, were as follows[1]:—

	Range	Mean
Daily max	94·4–105·6	100·2
Daily min.	63·0– 78·9	74·7
Daily mean	78·8– 92·3	86·4

For size-determinations of eggs the greatest diameter was measured by means of a calliper with dial graduations reading up to 0·1 mm. In the earlier stages (A and B) the diameter includes the egg-chorion, but after its rupture (Stage C onwards) the chorion was removed before measuring the eggs. This difference should be borne in mind in comparing the various stages, especially B and C.

Weighments were made in batches of 4 to 17 eggs each in a chemical balance reading up to 0·1 mg. The eggs, with or without the chorion, were cleansed of all dirt with a sable brush in sea-water, then washed with distilled water and the superfluous water quickly dried on a filter-paper. They were

[1] Data published with the permission of the Director-General of Observatories, Poona

then weighed in clean, dry glass dishes. For the determination of the water-content, drying was done in an air-oven at 105–110° C., the eggs being repeatedly weighed until the weight was constant. Before each weighing they were cooled over calcium chloride in a desiccator. Usually, the eggs took about 6–10 hours for complete drying. The chorion removed from the eggs was similarly treated for weighments. In the earlier stages (A and B) the eggs were weighed along with the chorion, in the later stages without it. The removal of the chorion was necessary as after rupture it accumulates, on the inside, droplets of superfluous water which are difficult to remove To obtain comparable readings in the computation of the final results, however, the mean weight of the chorion was added to the mean weight of eggs weighed without it.

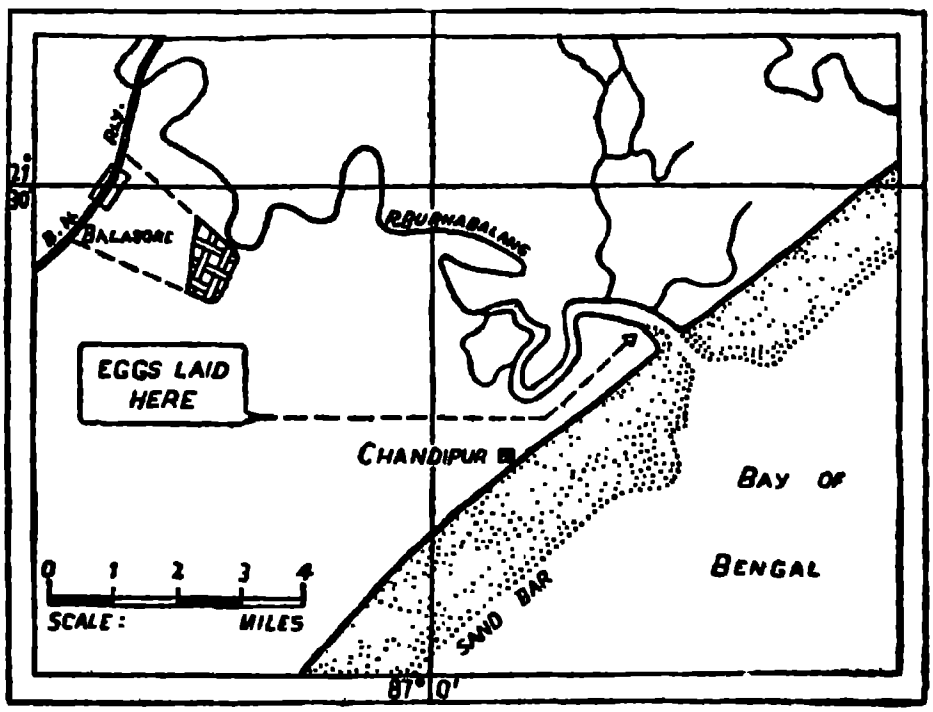
3. *Breeding Biology*

Very little is known of the breeding biology of *Tachypleus gigas*. Willemoes-Suhm (1883), on the testimony of local inhabitants in the Philippines and the East Indies, stated that the eggs both of *C. rotundicauda* and *T. gigas* are not laid in sand but are carried by the female on its abdominal appendages Annandale (1909) wrote similarly as follows (p 295) "On the coast of Bengal *L. moluccanus* [= *T. gigas*] breeds at the end of the cold weather, *i.e*, in March. The eggs, which are not very numerous, have a green colour and measure about 3 mm in diameter, are carried on the ventral surface of the abdominal appendages, to which they adhere lightly." These accounts have been accepted by subsequent writers including recent ones, *e.g.*, Gerhardt (1935, p. 87).

Iwanoff (1907–33), however, found in Java in 1906 that the eggs of *T. gigas* were laid almost throughout the year in sand in nests of some 200–300 eggs each. He explained (1933) that Annandale was probably led into error by accidentally expelled unfertilized eggs He added that frequently even fully ripe females, which sometimes carry about the male on their backs for several weeks, do not lay eggs for a long time apparently because they do not find the right places and other suitable conditions for egg-laying In such cases a certain number of eggs collect at the end of the genital ducts and around the genital opening at the hinder end of the operculum and remain stuck up there for some time. Such eggs remain unfertilized and do not develop.

The eggs studied by me were collected on March 8 and 9, 1941, near Chandipore (Balasore District, Orissa) in a tiny creek arising from the right bank of the R. Burhabalang close to its fall into the sea (Text-Fig 1) The creek was subject to tidal influence. At high tide it contained brackish water;

at low tide, when the eggs were collected, the mud in the creek was exposed This is, I believe, the only place where eggs have so far been found in India. Several hundred eggs were obtained some 2-3 inches beneath fine, dark-coloured mud. Each of the many egg-nests contained well over a hundred



TEXT-FIG. 1. Map of a part of the coastal region of Balasore District, Orissa, showing the approximate position where the eggs of *Tachypleus gigas* (Müller) were found on March 8 and 9, 1941 (From information kindly supplied by Dr B. N Chopra)

eggs. Among the dozen or so adults collected simultaneously, some copulating pairs were also obtained, but the ripe females were not seen to carry any eggs.

The preference for sand or mud for egg-laying is of some interest. In *T gigas* Iwanoff observed that in Java in the regencies of Batavia, Karwang and Bantam, with a total coastline of about 350 kilometres (218 miles), the only place where the eggs were laid was near the fishing village of Ssadari in the Karwang regency (N. E. Java). The eggs were laid in sand in a creek in the swampy mouth of the R. Ssadari, the creek being subject to tidal influence. The Java eggs were thus laid in a site exactly similar to that near Chandipore in India, with this difference that the former were laid deep in sand instead of mud. In the American King-Crab, *Xiphosura polyphemus*

(Linn.) too the eggs are laid in sand (Lockwood, 1870; Packard, 1872; Kingsley, 1892). In the Chinese King-Crab, *Tachypleus tridentatus* Leach [= *Limulus longispina* Van der Hoeven], Goto and Hattori (1929) found that in Japan the eggs were invariably laid in coarse sand, never in mud, but always in the vicinity of mud-flats.

Nothing is known of the actual process of copulation and egg-laying in *Tachypleus gigas* except Iwanoff's remarks, mentioned above, that the ripe females carry the males on their backs. The following notes, made on March 4 and 5, 1939, by Drs. B. N. Chopra and H. S. Rao at the Chandipore sea beach in the neighbourhood of the same place where the eggs described here were found in 1941, are taken from one of the Field Station Books of the Zoological Survey of India, and are of some interest. Although I have not been able to examine the specimens that they collected, the mention of the comparative development of the spines in the adult ♂♂ and ♀♀ leaves no room for doubt that the species in question was *T. gigas* and not *C. rotundicauda* (*cf.* Pocock, 1902). The animals were observed on the open intertidal beach which consists of firm mud and sand; no eggs were found.

> "A few specimens of *Limulus* buried in sand (nearly always in pairs), some of them carrying sea-anemones and barnacles[2] were found near the fishermen's traps The pairs always consisted of the smaller one riding on the back of the larger (probably the female), holding on to the abdominal part of the larger specimen by its first two pairs of eggs to the last two pairs of the lateral spines of the abdomen There seems to be some variation in the manner of the smaller individual attaching itself to the larger and also in the reduction of the abdominal spines, Nos. 4 to 6 The 4th and 5th spines of the abdomen of the larger specimen are reduced. All the six spines of the smaller specimen are normally long. The actinians and barnacles are found usually on the smaller specimens, even covering the eyes in some cases.[3] When the two are separated forcibly and placed on dry land the larger one moves down to the water while the smaller appears to be helpless and does not usually follow its mate. Placed close together, however, the smaller gets on to its normal position over the back of the larger.

[2,3] *Cf.* Shipley's (1909, pp. 260–61) account of *Limulus polyphemus* "The whole body is covered with a smooth chitinous sheath varying from saga-green to black in colour, and it is kept very clean, probably by some excretion which hinders various sessile animals attaching themselves to it as they do, for instance, on many Copepods. Burrowing animals like *Limulus* are usually free from these messmates. King-crabs have a self-respecting well-groomed appearance."

They are locally known as *Ram-Lakhan magar*,[4] the bigger one being *Ram* and the smaller *Lakhan*."

4. Developmental Stages of Eggs

The incubation period of *Tachypleus gigas* is not known, but Iwanoff (1933), by comparison with other species of king-crabs, surmised it to be somewhat more than 6 weeks. The age of the eggs collected near Chandipore varied from those that were apparently recently laid to those that were comparatively advanced in development (with the egg-chorion ruptured, *i.e.*, some 2 or 3 weeks old). In each nest the eggs were, on the whole, at about the same stage of development, and this condition provided a safe and easy method of obtaining progressive stages of development. As the eggs were collected on March 8 and 9 and the latest date observed for the rupture of the chorion was March 25, the latter event evidently took place at least 17–18 days after oviposition. Thus, the rupture of the chorion, which occurs in about the third week after oviposition or after the completion of about one-third to one-half of development, provided a good point of reference for all the stages. The beginning of embryonic movements provided another such point. On these bases, nine stages, A–I, are recognised in the present account, as follows:—

A (Pl. III, Fig. 1).—Young, greenish eggs, roughly in 2nd week after oviposition—collected on March 8 and examined on March 17. No formed embryo visible on dissection. Several days before rupture of egg-chorion.

B.—Shortly (a few hours) before rupture of chorion Eggs roughly in 3rd week of development. Embryo small but well formed.

C. (Pl. III, Fig. 2).—Shortly (a few hours) after rupture of chorion. About 1 day older than B. Embryo dimly seen through translucent *cuticula blastodermica.*

D.—One day after rupture of chorion.

E.—(Pl III, Fig. 3).—Two days after rupture of chorion. No embryonic movements visible.

F.—Three days after rupture of chorion. First signs of embryonic movements visible, the legs moving feebly; no rotations yet seen.

G.—(Pl. III, Fig. 4).—Five days after rupture of chorion. Leg movements vigorous. Embryonic rotations well on the way, having started on previous day, *i.e.*, 4th day after rupture of chorion.

[4] The allusion is to the two brothers, the elder *Rama* and the younger *Lakshmana*, who are the heroes of the ancient Hindu epic, *Ramayana*. *Magar* in Hindi means a crocodile.

H.—(Pl. III, Fig. 5).—Nine days after rupture of chorion. Embryo rotating vigorously.

I.—Older than H—about 12 days after rupture of chorion. Embryonic rotations vigorous, showing both somersaulting and rolling. Moulted cuticle floating inside egg by side of embryo.

5. *Colour, Shape and Size of Eggs*

(Pl. III. Text-Fig 2 and Tables I, III and IV)

Colour and Shape.—The young eggs (Stage A) are rounded and somewhat resemble the seeds of *bhindi* or lady's finger (*Hibiscus esculentus*) in shape and size and partly also in colour. They are irregularly polyhedral evidently owing to mutual pressure, and are pale olive-grey throughout: in a few eggs opaque whitish patches are visible. The eggs are covered by a thick, colourless, leathery, translucent and structureless chorion which, when it ruptures later, is seen to consist of more than one layer In Stage B the eggs have somewhat increased in size but are otherwise unchanged. In Stage C the egg-chorion has ruptured, exposing the thin *cuticula blastodermica*[5] which now surrounds the egg. As the egg swells, the single straight slit in the chorion widens and additional slits may develop, until the chorion is split up into two or more pieces which remain on the egg for a long time (Stage I), the various chorionic pieces generally remaining attached to one another by small portions of the chorion Owing to wetness they probably remain attached to the egg until hatching After the rupture of the chorion the eggs become more or less spherical owing to turgescence caused by the intake of water, but there remains in some eggs an appreciable difference between their smallest and the greatest diameters. Thus, of a few eggs measured in Stage I, the largest and the smallest eggs gave the following figures respectively: $6{\cdot}7 \times 6{\cdot}2$ and $6{\cdot}5 \times 6{\cdot}0$ mm.

Size.—Freshly laid eggs were not available to me, but Iwanoff (1933) gave their maximum diameter as 3·35 mm. (mean 3·25 mm., which gives a volume of about 18 cu. mm.) The range of variation in the greatest diameter of the egg within each stage is given in Table I. Notwithstanding certain overlapping in the individual figures, the means clearly indicate a

[5] The *cuticula blastodermica* (deutovum of some old authors) is not strictly an egg-wall as it is secreted long after oviposition by the embryonic cells. It occurs also in other species of king-crabs. Iwanoff (1933), in *T. gigas*, called it the primary or first cuticle. He found that as development proceeds, the embryo secretes two more extremely thin cuticular membranes, the tritovum and the tetratovum, which are after a time moulted and are seen floating inside the egg.

gradual increase of size with development. In Stage A the mean diameter is 3·57 ± 0·1493 mm., and in B 3·86 ± 0·1449, or a mean increase of only 8·1%. Between B and C there is no apparent increase, but since C, unlike B, was measured without the egg-chorion (which ruptures), a slight increase evidently occurs. In subsequent stages the increase is so great that correction for chorion-width may be ignored. The increase from C to I is 73·3%. The total increase between Stages A to I is 3·12 mm. or 87·4%.

Since volume gives a better idea of size-increase than diameter, it was calculated from the latter by the formula $\frac{4}{3}\pi r^3$ (where r is the radius) on the assumption that the eggs are perfectly spherical (Tables II—IV). As already stated, the eggs are irregularly spherical. They are roughly polyhedral in the early stages (A and B), and roughly spherical or ellipsoidal in the later ones (C–I) after the rupture of the chorion. The general conclusions for volume are the same as for diameter. The total volume increase between Stages A–I is from 23·8 to 156·8, *i.e.*, 133 cu.mm. or 558·8% (Table III).

TABLE I

Measurements, etc., of the greatest egg-diameter (mm.)

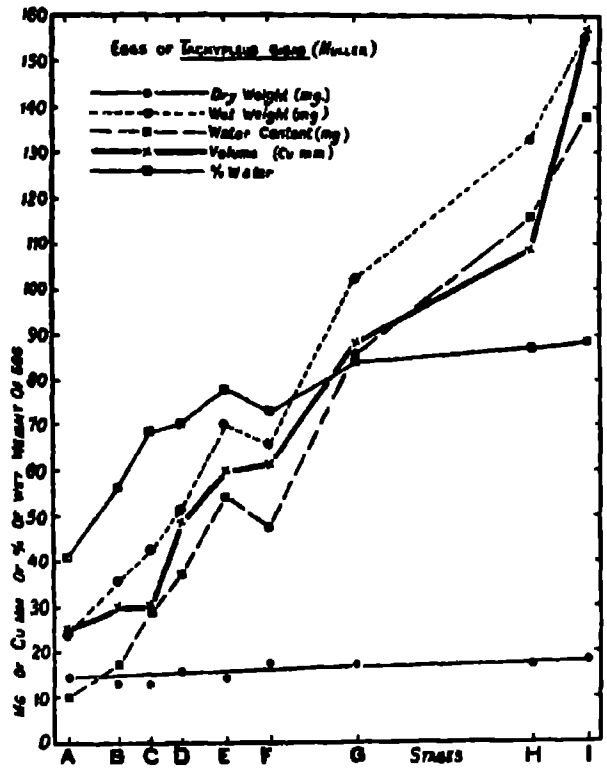
(Stages A and B measured with chorion ; C–I without it.)

Stage and number of eggs measured	Greatest diameter (mm.)			
	Range	Arithmetic Mean	Standard Deviation	Standard Error of Mean
A (93)	3·3–3·9	3·57	0·1493	0·0155
B (40)	3·6–4·2	3·86	0·1449	0·0229
C (15)	3·7–4·2	3·86	0·1549	0·0399
D (8)	4·3–4·7	4·54	0·1507	0·0532
E (5)	4·7–5·1	4·86	0·1673	0·0748
F (9)	4·7–5·2	4·90	0·1732	0·0577
G (9)	5·4–5·7	5·52	0·1039	0·0346
H (19)	5·7–6·3	5·93	0·1694	0·0389
I (14)	6·5–7·3	6·69	0·2657	0·0710

Discussion.—As will be evident from the above account, with development the egg swells considerably, especially after the rupture of the chorion. The increase of wet weight and water-content show conclusively that the swelling is due primarily to the intake of water largely through the *cuticula blastodermica*, and to a lesser extent through the egg-chorion. Such a swelling is known to occur in all the species of king-crabs studied, but, as far as I know, actual size-measurements (except of freshly laid eggs), weights, etc., were hitherto not available for any species.

From freshly laid eggs (Iwanoff's data) to Stage A the volume increases from 18 to 23·8 cu. mm. or by 32·2%. From A to B the increase is 26·5%.

Thus the total increase up to the rupture of the chorion (Stage B), *i.e.*, during nearly the first 3 weeks of development, is 58·7%. After rupture and the consequent exposure of the thin and highly permeable *cuticula blastodermica* the increase in a single day (Stage C to D) is 62·8%. Subsequently, the increase slows down, being only 22·7% on the following day (Stage D-E). The average daily increase for the 11 days from Stage D to I is only 20%.



TEXT-FIG. 2. Graph showing the volume (cu. mm.), dry and wet weights (mg.) and water content (mg and as % of wet weight) of the egg of *Tachypleus gigas* (Müller) in various developmental stages (A–I) (Distances between some of the stages on the abcissa are approximate.)

Iwanoff (1933, pp. 170–173) noted the following two kinds of swellings in *T. gigas*:—(i) 2–3 hours after fertilization (which presumably occurs immediately after oviposition) the eggs swell apparently, according to Iwanoff, under the influence of the change of permeability of their outer wall due to fertilization and the swelling of the egg-plasma. The degree of swelling is not mentioned by Iwanoff. I was unable to make any observation regarding this swelling. (ii) "At the stage when the chorion ruptures, *i.e.*, the inner egg-membrane or so-called cuticula blastodermica first begins to

swell, and detaches itself from the egg-surface, many embryos die—very likely as a result of the onset of new osmotic conditions and demands which might have been brought about by the rearing of the eggs in sand moistened with fresh-water. On the other hand, in the stages following the swelling of the cuticula blastodermica, during which the egg visibly increased in volume, the dying of the embryos completely stopped and their development continued to be entirely normal until the hatching of the larva." No further details are given by Iwanoff My observations are somewhat different. Firstly, while egg-swelling is clear, it occurs even before the rupture of the chorion, though the degree of swelling after rupture is much greater. Secondly, Iwanoff seems to imply, if I understand him correctly, that the *Cuticula blastodermica* swells up soon after the chorionic rupture. Actually the cuticle itself does not swell, but stretches and becomes thinner owing to the increase in egg-volume consequent upon the rapid water-intake after the chorionic rupture Thirdly, the many deaths reported by Iwanoff did not occur in my rearings owing evidently to my employment of sea-water for moistening the eggs.

6 *Weight and Water-Content of Eggs*

(Text-Fig 2 and Tables II–IV)

Weight of Egg-chorion —Since some eggs were weighed with the chorion and others without it, it became necessary, in order to obtain comparable readings, to make corrections for the weight of the chorion Seven complete chorions from eggs in Stage I were washed, dried on a filter-paper and their wet and dry weights determined as for the eggs. The following mean weights were obtained for one chorion:

Wet wt . 2·60 mg.

Dry wt. · 2·09 mg

Loss (= water content): 0·51 mg. (or 19·2% of wet wt.)

These weights were added to the mean weights of single eggs weighed without chorion, it being assumed that the chorion-weight obtained for Stage I applied equally to the other stages. Example (Stage C, Expt. 14):

Mean wt. of one egg without chorion: Wet wt. 40·02 mg.

Dry wt. 11·30 mg.

Corrected mean wt. of one egg (wt. of chorion added):

Wet wt.: 40·02 + 2·60 = 42 62 mg.

Dry wt.: 11·30 + 2·09 = 13·39 mg.

Loss (= water content): 29·23 mg. (or 68·6% of wet wt.).

TABLE II. *Wet and dry weights (in mg.) and percentage of water in eggs in various experiments*

Stage	Expt. No	No of eggs weighed in each expt	Average wt. of one egg (eggs weighed without chorion)		Average wt. of one egg (eggs weighed with chorion or corrected for chorion weight where not so weighed)		Average loss in wt. (=water-content in mg.) in one egg (corrected, where necessary, for weight of chorion) Based on columns *f* and *g*	% loss in wt. (=water-content) in terms of wet wt. of egg
			Wet wt	Dry wt	Wet wt	Dry wt.		
a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*
A	1	10			23·10 } Mean	14·58 } Mean	8·52 } Mean	36·9 } Mean
A	2	10			23·42 } 24·17	13·98 } 14·20	9·44 } 9·97	40·3 } 41·1
A	6	10			26·00 }	14·05 }	11·95 }	45·95 }
B	13	17			30·58	13·56	17·02	55·7
C	14	4	40·02	11·30	42·62	13·39	29·23	68·6
D	15	14	48·86	13·31	51·46	15·40	36·06	70·1
E	9	3	67·40	12·73	70·00	14·82	55·18	78·8
F	3	10	63·40	15 70	66·00	17·79	48·21	73·1
G	10	5	99·76	14·16	102·36	16·25	86·11	84·1
H	5	10	131·00	15·76	133·60 } Mean	17·85 } Mean	115·75 } Mean	86·6 } Mean
H	11	4	130·35	14·90	132·95 } 133·28	16·99 } 17·42	115·96 } 115·86	87·2 } 86·9
I	12	9	153·62	15 93	156·22	18·02	138·20	88·5

TABLE III. *Summary of means, etc., of one egg regarding diameter, volume, weight, etc., and percentage increase*

(Diameter in stages A and B measured with chorion, C–I without it.)

Stage	Greatest diameter (mm.)	Calculated volume (cu. mm)	Wet wt. (mg.)	Dry wt (mg.)	Loss in wt. (=water-content)		% increase over previous stage			
					In mg.	% of wet wt	Greatest diameter	Calculated volume	Wet wt.	Water-content
A	3·57	23·8	24·17	14·20	9·97	41·1	..	..	..	..
B	3·86	30·1	30·58	13·56	17·02	55·7	8·1	26·5	26·5	70·1
C	3·86*	30·1	42·62	13·39	29·23	68·6	(0)*	(0)*	39·4	71·7
D	4·54	49·0	51·46	15·40	36·06	70·1	17·6	62·8	20·7	23·4
E	4·86	60·1	70·00	14·82	55·18	78·8	7·1	22·7	36·0	53·0
F	4·90	61·6	66·00	17·79	48·21	73·1	0·8	2·5	(−5·7)	(−12·6)
G	5·52	88·1	102·36	16·25	86·11	84·1	12·7	43·0	55·1	78·6
H	5·93	109·2	133·28	17·42	115·86	86·9	7·4	24·0	30·2	34·5
I	6·69	156·8	156·22	18·02	138·20	88·5	12·8	43·6	17·2	19·3
Total increase A—I	3·12	133·0	132·05	3·82	128·23	115·3	87·4	558·8	546·3	1186·2

* See text, p. 122.

Weight and Water-content of Eggs.—The mean wet weight of an egg increases from 24·17 mg. in Stage A to 30·58 mg. shortly before the rupture of the chorion (Stage B), *i.e.*, by 26·5%. With the rupture of the chorion and the consequent rapid intake of water the mean wet weight increases to 42·62 mg. (or by 39·4% over B) within a few hours (Stage C). Then onwards there is a steady in crease of wet weight which in Stage I is 156·22 mg. There is a curious and rather problematical fall of about 5·7% between Stages E and F. The total increase between Stages A and I is 546·3% or nearly 5½ times.

The mean dry weight of an egg shows an increase from 14·20 mg. in Stage A to 18·02 mg. in Stage I, or by 26·9%. The figures are rather irregular (Text-Fig. 2) and occasionally show a slight decrease. This irregularity, however, is probably not real, and, on the whole, a steady increase in dry weight may be said to occur. Unless the figures of the entire embryonic period are available, it is of course impossible to say whether the increase continues until the end. In some insects, *e.g.*, locusts (Roonwal, 1936) there is a decrease of about 20% in the dry weight of the egg-material during embryonic development.

The water-content of an egg, as judged by the difference between the wet and dry weights, shows an almost parallel tendency to the wet weight. Between Stages A and B the water increases from 9·97 to 17·02 mg., or by 70·1%. With the rupture of the chorion, the water increases in a few hours (Stage C) to 29·23 mg., or by 71·7% over B. Then onwards there is a steady increase (except for a problematical fall between Stages E and F) to 138·20 mg. in Stage I. The total increase of water between Stages A and I is 128·23 mg. or 1186·2%, *i.e.*, nearly 12 times.

In Stage A the water is about 41·1% of the wet weight of the egg, in B 55·7%, in C (shortly after chorionic rupture) 68·6% and in I 88·5%. The total increase from A to I is 115·3% or, in other words, the relative weight of water is more than doubled.

It is thus clear that the swelling and the increase in wet weight of the egg with the progress of development is due almost entirely to the intake of external water, the increase in dry weight being extremely small.

Relation between Weight and Volume of Eggs (Table IV).—The correspondence between wet weight, volume and water-content of the eggs is fairly close in most stages and a nearly parallel increase occurs in all the three features. However, between Stages B and C there is little volume-increase though the weight increases considerably; and between E and F there is a problematical decrease in wet weight though the volume continues to increase.

The ratio W/V, where W is the mean wet weight (in mg.) and V the mean calculated volume (in cu. mm.) of an egg, is always somewhat above unity (Table IV) except in the last Stage, I, where it is 0·996 The ratio is highest in Stage C, being 1·42.

TABLE IV

Ratio W/V (mean wet weight of an egg to mean volume)

Stage	A	B	C	D	E	F	G	H	I
W/V	1·02	1·02	1·42	1·05	1·16	1·07	1·16	1·22	0·996

7. Embryonic Movements

When the egg-chorion ruptures (Stage C), the embryo can be dimly seen through the thin cuticula blastodermica. This cuticle remains more or less translucent until Stage E, but with increasing swelling of the egg it stretches and becomes transparent. In Stage E the embryo is seen floating freely in a clear fluid but does not as yet show any movements. In Stage F (3 days after chorionic rupture) the first feeble movements of the legs are noticeable. A day later the leg movements are more vigorous and some of the embryos are seen to undergo extremely slow rotations along the antero-posterior axis of the body, the head always going down first; these may be called " front rotations " for brevity. In others there are no signs of rotations. In Stage G (5 days after rupture) the legs move vigorously and embryonic rotations are well on the way though still somewhat irregular and slow. The *cuticula blastodermica* is now quite transparent. On the sixth day after rupture the embryo is seen to rotate quite vigorously. In Stage H (9 days after rupture) the embryo rotates vigorously and almost continuously, each full front rotation taking 5–10 seconds. Three days later (Stage I) it is seen that rotations are not on a single axis. Sometimes the embryo rotates, as before, along the antero-posterior axis (front rotations); at others, along the right-left axis (side rotations). The two kinds of rotations occur in the same embryo without any evident regularity. By this time the embryo has undergone a moult and the crumpled exuviae are seen floating in the egg-fluid. On the thirteenth day after rupture the embryos rotate more vigorously than before, each front rotation now taking 3–8 seconds. On the fourteenth day after rupture the embryonic movements became sluggish, owing apparently to the onset of some pathological condition, and 3 days later all the embryos unfortunately died.

As far as I could observe, the mechanism of the rotations is somewhat as follows. The legs strike against the *cuticula blastodermica* and propel

the embryo either forwards or sideways depending on the kind of rotation performed. The egg-fluid in which the embryo floats freely no doubt facilitates the rotations. I could observe no pulsatile or peristaltic movements of the embryo as occur, for example, during the single and very slow rotation (blastokinesis) of some insects, *e g.*, locusts and grasshoppers (Slifer, 1932; Roonwal, 1937), where the rotation takes about a day or more (17–20 hours at 33° C in *Locusta*).

8. Summary

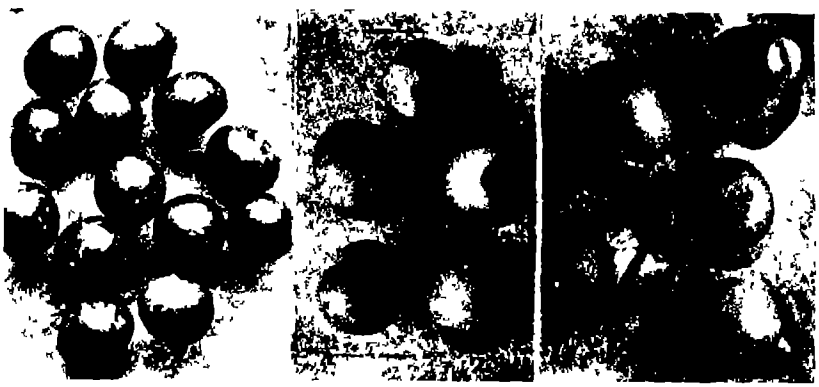
1. Several hundred eggs of *Tachypleus gigas* (Müller) [- *Limulus mollucanus* Latr.] were obtained on March 8 and 9, 1941 near Chandipore beach (Balasore District, Orissa, India) in a tiny creek arising from the R Burhabalang close to its fall into the sea. They were brought over to Calcutta and studied alive for nearly a month which probably covers about one-half to two-thirds of the incubation period. Some 9 development stages, A–I, were recognized for reference.

2. The eggs were laid in mud in the tidal zone in nests of over 100 eggs each. They were not carried on the abdominal appendages of the ♀ ♀.

3. The eggs are nearly spherical. They are first bounded by a thick, leathery chorion which later ruptures, exposing a thin, elastic *cuticula blastodermica*. Their greatest diameter was measured and the volume calculated therefrom. The mean diameter of young eggs is 3·57 mm. in Stage A (2nd week of development), 3·86 mm. in C (shortly after the rupture of the egg-chorion) and 6·69 mm. in I or nearly double of the original in Stage A. The volume increases nearly 5½ times. The swelling is due to the intake of water by the eggs.

4 Between Stages A and I the mean wet weight of an egg increases from 24·17 mg. to 156·22 mg., or nearly 5½ times. The mean dry weight rises from 14·20 to 18·02 mg., or by 26·9%. The water-content rises from 9·97 to 138·20 mg, or nearly 12 times, the greater part of the increase occurring after the rupture of the chorion.

5. Embryonic movements, which begin in Stage F, consist at first of feeble leg movements which gradually become vigorous and are accompanied by continuous rotations along the antero-posterior and right-left axes of the embryo. The rotations are apparently caused by leg movements, and are not due to peristalsis or pulsations of the embryo.



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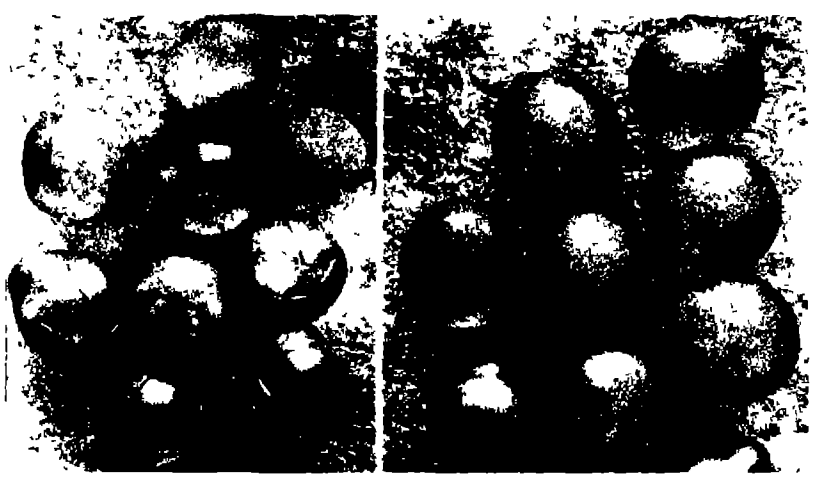

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Photographs of eggs of *Tachypleus gigas* (Muller), showing increase of size as development proceeds. ½

FIG. 1 *Stage A*. Young greenish eggs roughly in 2nd week after oviposition. No formed embryo visible. Eggs opaque owing to thick, leathery chorion. Note that several eggs are irregularly polyhedral.

FIG. 2 *Stage C*. Shortly (a few hours) after rupture of egg-chorion (*cf.* Fig. 1). The eggs are now more or less spherical. The ruptured chorion is seen attached to all the eggs.

FIG. 3 *Stage E*. ~2 days after rupture of chorion. Embryonic movements not yet begun. The splitting of chorion has progressed much farther.

FIG. 4 *Stage G*.—5 days after rupture of chorion. Embryonic movements vigorous.

FIG. 5 *Stage H*.—9 days after rupture of chorion. Embryo rotating vigorously. Chorion still attached to a few eggs.

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ON THE SUBMARINE VOLCANIC ORIGIN OF ROCK-SALT DEPOSITS

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Rock-salt is one of the commonest of minerals occurring widely distributed in the world. It is also known to have been deposited in different epochs of the earth's history. The problem of the origin of rock-salt has long been the subject of discussion among geologists but no satisfactory solution has yet been found The general consensus of opinion, however, appears to be that salt deposits have been formed somehow through evaporation of saline waters

The sedimentary hypothesis of rock-salt formation is probably the most obvious one since the sea is the largest repository of salt on the earth's surface. Sea water contains about 3·5 per cent. saline matter in solution. Of this, common salt forms the major portion and is estimated, in its total quantity, to exceed ten thousand billion tons.

Many lakes are highly saline and appear to be actually depositing salt layers on their beds.

Further the sedimentary origin of salt is indicated by the bedded nature of some salt deposits as also by their nearly constant association with stratified rocks.

From all these considerations it is now generally held that salt deposits owe their formation to evaporation of saline waters in sea or lake basins. The controversy which, however, still exists pertains chiefly to the actual physical conditions obtaining during the process of evaporation.

The Bar Theory of Ochsenius

The difficulty of visualising evaporation of waters in an open sea ever reaching the stage of precipitation led C. Ochsenius, to advocate deposition of salt in oceanic bays separated from the open sea by a level bar. Continuous evaporation would increase the salinity of the bay waters whereas the decrease in volume of waters in the bay would be compensated by continuous or intermittent flow of sea water over the bar. Dry warm climate and absence of fresh water tributaries to the bay were postulated as essential conditions obtaining during this process.

Increase in salinity through evaporation increased the density of the surface solutions which sank to deeper levels while fresh accession of sea water was being made at the surface. The total salinity of the bay increased gradually till saturation was reached when salt began to precipitate at the bottom.

Some Difficulties of the Bar Theory

In its fundamental principles the Bar theory as elaborated by Ochsenius furnished a possible explanation of the formation of salt deposits in sea basins whereas the classical investigations of van't Hoff and others on the Stassfurt deposits demonstrated the theoretical grounding of the chemistry of the process operating during salt formation.

It is however when we consider the physical conditions postulated by Ochsenius as essential for the process that we meet with some difficulties

Since the total salt content of sea waters is only 3·5% a very large volume of water must evaporate before a thin layer of salt could get deposited. The influx of sea water at any time cannot be very large and for the deposition of any moderate thickness of salt a very large number of periodical influxes must be postulated. Beds of rock-salt over 1,000 ft. thick are not at all uncommon and to account for these an undisturbed repetition of evaporation-influx process must be presumed to have continued for long geological periods.

In this connection it has to be pointed out that the bar which brought about separation of the bay from the sea is by itself a very evanescent physical feature and cannot survive for the long geological time necessary for the formation of thick salt beds. The undisturbed continuity of physical and climatic conditions, *e.g.*, absence of fresh water tributaries, dry warm climate, maintenance of bar level preventing the bay waters flowing seaword, etc., for long periods is also difficult to visualise

In a deposit formed in the manner indicated above the proportion between the various saline constituents and particularly between sodium chloride and calcium sulphate in the sea water must be maintained at least roughly in the resultant precipitates.

This relation is rarely met with in the deposits.

Salt deposits are often found in very high latitudes which have temperate or even cold climates In these regions evaporation must be an extremely slow process and cannot, by itself, account for the formation of thick salt beds.

Besides halite the salt deposits also contain a number of other minerals some of which are typical of high temperature paragenesis. Among these may be mentioned the Hartsalz[1] Paragenesis typically observed in the Carnellite zone of the Stassfurt salt deposit. The minimum temperature of the formation of this paragenesis[1] Sylvine, Kainite, Carnellite, Halite and Kieserite, is 72° C. or about 130° F. Very few regions on the earth's surface ever reach such high temperatures. No oceanic basins can ever attain this temperature except in the vicinity of active volcanoes. It is therefore very difficult to account for the formation of such deposits through simple surface evaporation of oceanic or even inland basins.

These are some of the points which make the Bar theory not wholly acceptable in accounting for the formation of large salt deposits.

Theories of Continental Deposition—Other Sedimentary Theories

The Bar Theory of Ochsenius was sharply criticised by J. Walther, E Erdamann and others. Walther,[2] from the common occurrence of rock-salt and salt lakes in deserts, explained the salt formation by lixiviation of the saline contents of the sedimentary rocks and accumulation and evaporation of the resulting solutions in desert basins, where conditions are most favourable for rapid evaporation. The main difficulty with this theory is that there is not enough circulating or flowing water in these rainless deserts to bring about either rock decomposition or leaching of saline matter from surrounding rock formations which are generally very poor in salt content. The desert streams are therefore incapable of bringing about accumulation of salt sufficient to make the lakes highly saline unless salt occurs autochthonous in the drainage area of the lake.

I. C. Russel[3] advocated the Desiccation Theory according to which former inland lakes on extreme evaporation deposited considerable quantity of salt on their bed. According to him salt got absorbed by lacustrine clays during desiccation while the next monsoon brought fresh quantity of salt to the lake. This process was repeated and after a long period of concentration the lake waters became strongly saline and finally evaporated to dryness leaving a deposit of salt over the lake bed.

These Lixiviation and Desiccation Theories may account for a few small occurrences but are generally not applicable to larger deposits.

[1] U. Grubenman and P. Niggli, *Die Gesteinsmetamorphose*, 1924, 46, p. 142.

[2] J. Walther, *Das Gesetz der Wustenbildung*, Berlin, 1900.

[3] "Salt Resources of the United States," *U. S. Geol. Sur. Bull.*, 1919, No. 669.

Hypogene Origin of Rock-Salt

The fact that sodium chloride and hydrochloric acid are among the important constituents of volcanic emanations has led several geologists to assign a volcanic origin to salt beds. Some even hold that most of the saline contents of the sea are primarily of volcanic origin.

In a number of deposits salt occurs in the form of domes and plugs as in Persia,[4] Algeria, Tunisia, Andalucia, Pyrenees, Mexico, United States, etc., where salt is observed to show intrusive relations with the associated stratified formations. The salt in these 'intrusive' bodies does not show any bedding. The junction of the salt deposits with the adjoining rocks, instead of being sharp is very commonly irregular and brecciated, the fragments of associated rocks being often found enclosed within the salt deposit. This has led many investigators to assign an igneous origin to these deposits.

Middlemiss,[5] discussing the origin of salt marl in Salt Range, visualised it as a scum secreted at the surface of an ancient untapped magma which on intrusion or injection sometime during Tertiary Period consolidated as marl.

The most usual association of salt and gypsum which is commonly held as truly sedimentary could be equally of igneous origin since SO_3 which is a common volcanic exhalation would, on reaction with limestone, easily form gypsum. It is frequently seen that the two minerals occur in different proportions in different parts of the salt deposit and form irregular masses, quite unlike what one would expect from a normal precipitate formed through the simple process of evaporation.

There are again several other facts concerning association which are not easily explained on the basis of the evaporation theory but which would be normally expected on volcanic theory.

Among such evidences may be mentioned the very frequent association of volcanic rocks, ashes and tuffs as in the Carpathian mountains, United States, Persia, Salt Range, etc.

Sulphur and sulphurous gases as also the borates and nitrates are common products of volcanic activity. Their occurrence in salt beds lends a strong support to the view that volcanic activity had some significant part to play during the formation of these deposits.

[4] J. V. Harrison, "Geology of Some Salt Plugs in Laristan," Q. *J. G. S.*, Vol. 86, Pt. 4, pp. 463–552.

[5] *Rec. G. S. I.*, Vol. 24, pt. 1, p. 42.

If however salt were only of igneous origin the occurrence of salt deposits should have been restricted to the immediate vicinity of igneous intrusions, a feature, which is comparatively less frequent. On the other hand the deposits are most commonly associated with such sedimentary rock formations where volcanic rather than intrusive igneous activity has played a definite but minor part.

It would then appear that salt deposits show some features which are characteristic of sedimentary deposition but they also show others which are peculiar to igneous origin. No one mode of formation can by itself explain all the characteristics of salt deposits and it would therefore be necessary to visualise a combined process where both sedimentation and igneous action play their respective roles.

Geological and Geographical Distribution of Rock-Salt Deposits

In order to arrive at a proper understanding of such a comprehensive process it may be helpful to study, in general the geological and geographical distribution of rock-salt deposits of the world.

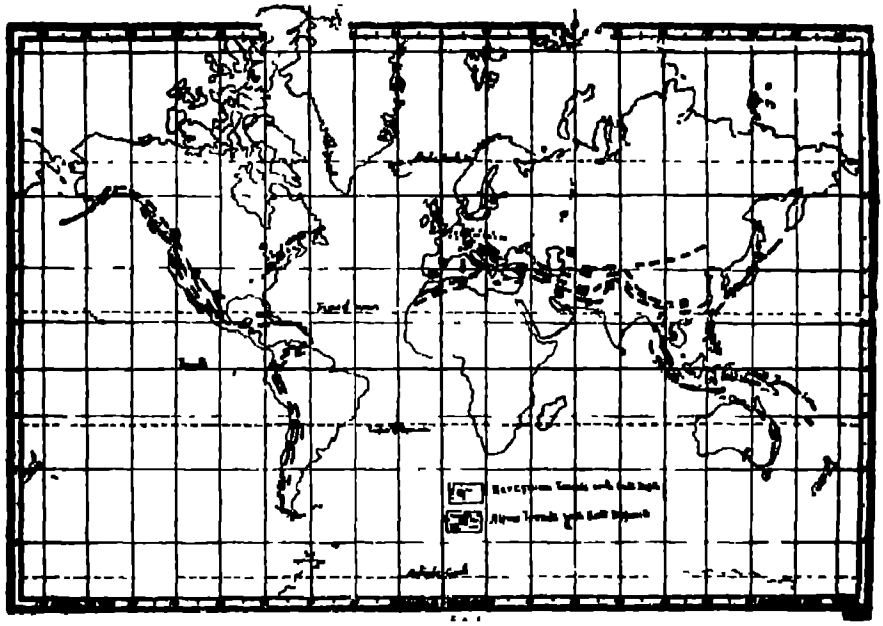
Rock-salt is known to be associated more or less with rocks of all ages right from Cambrian down to Recent. Cambrian age has been attributed to the salt deposits of the Punjab Salt Range, and of Persia. This age, however, has been seriously questioned.

Salt deposits are known from Upper Silurian formation (Onondago Salt Group) of New York State, Michigan and Ontario where they are associated with red marl and gypsum.

A Carboniferous age has been assigned to the salt deposits of Nova Scotia and Montana in United States and to those of Kimberley District of Western Australia.

Permo-Trias is the period of intense salt formation and some of the largest rock-salt deposits are found associated with formations of this age in Central Germany, Russia, southern Scotland and the eastern and central parts of the United States (Fig. 1). More than half of the world's annual output of rock-salt comes from these deposits. It may be remarked here that the region extending from eastern United States on the west to the Urals on the east forms a zone of Hercynian mountain activity and that the salt beds in this zone appear to be intimately connected with this late Palæozoic orogenesis.

Leaving the Westphalian rock-salt deposits of Germany which are associated with upper Jurassic (Purbeck) limestones, the next important period of salt formation is the Tertiary when rock-salt got extensively



deposited all along the western sea board of the Americas from Br. Columbia in the north to Chile in the south and also along the Atlas ranges, Spanish Meseta, the Carpathian and the Caucasus ranges, the Aral-Caspian depression, Turkish and Persian ranges extending up to the Punjab Himalayas. Tertiary rock-salt also occurs in parts of China and Thailand.

From this distribution it is quite obvious that the Tertiary rock-salt has closely followed the trends of the Tertiary orogenesis represented by the Andean and the Alpine-Himalayan mountain systems (Fig. 1) In several regions these salt formations have been intricately involved in the mountain folding while they are also seen at places to be intimately associated with eruptive sheets.

In post-Tertiary period there are no indications of extensive rock-salt formation; the few stray deposits found associated with saline lakes in the present desertic regions, *e.g.*, Sambhar lake in Rajputana, India, appear to the author to be formed largely through solution and reprecipitation of Tertiary or older salt deposits found *in situ* in these deserts.

From this review it would be seen that the rock-salt formation is closely related, both in geological time and geographical distribution, to the orogenic upheavals particularly of the Hercynian and the Alpine movements, both of which are accompanied by extensive volcanic action.

Theory of Submarine Igenous Activity

The Theory of Salt Formation suggested here is based on the appreciation of the fact that a preponderant number of Tertiary salt deposits of the world are confined to a narrow belt running parallel to, and closely following on either side, the Alpine-Himalayan and the Andean chain of mountains. These salt deposits represent nearly the last marine sediments (precipitates) deposited in the Tethyan seas. These salt beds have further been involved in the complex orogenic folding which the region has undergone. There is therefore a possibility that the salt formation particularly of the Tertiary period marks a closing event in the depositional history of this Mesozoic geosyncline.

It may be presumed here that for millions of years during the geosynclinal phase the Tethys was continually receiving sediments and saline constituents from the continental regions (Fig. 2*a*). The suspended and the

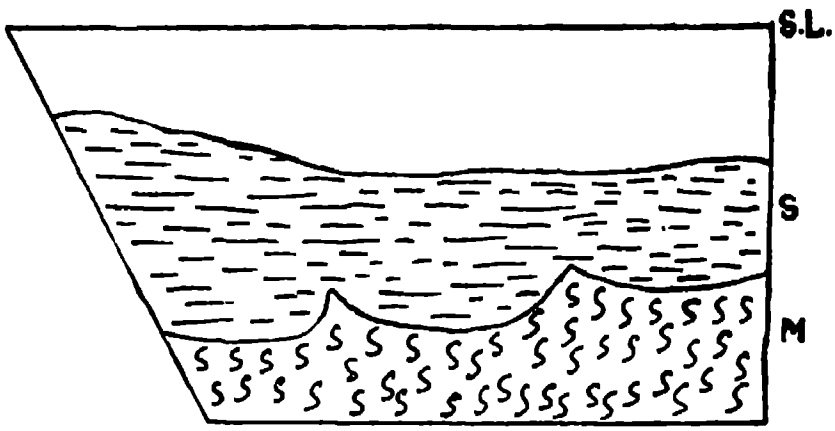


FIG 2*a*. Geosynclinal Phase

M., Metamorphic basement ; *S*., Continuous Deposition of Sediments

less soluble materials got deposited, but the more soluble ones like sodium chloride remained behind in solution The sea water was thus constantly, though inappreciably, getting richer in certain dissolved salts.

Towards the close of the marine period when due to orogenic movements the sea floor with its thick series of sediments got heaved up and thrown into folded wrinkles (Fig 2*b*) the Tethys Sea was diminishing in depth and extent and at certain periods accommodated itself in detached longitudinal synclinal basins (Fig. 2*c*). It is these basins which appear to have served as sites for the formation of large Tertiary salt deposits.

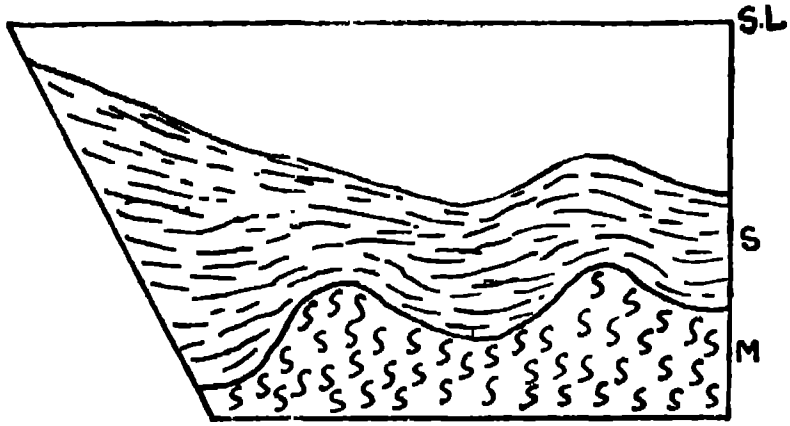


FIG. 2*b*. Embyronic Orogen
Gentle folding in bedded deposits

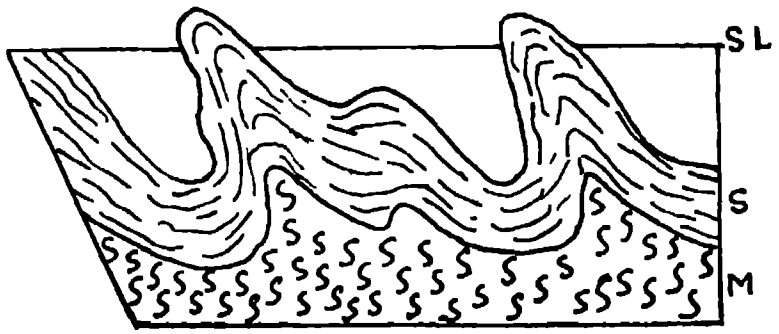


FIG. 2*c*. Mid-Orogen
Intense folding and upheaval ; shallowing and partitioning of the Geosynclinal sea into separate longitudinal basins

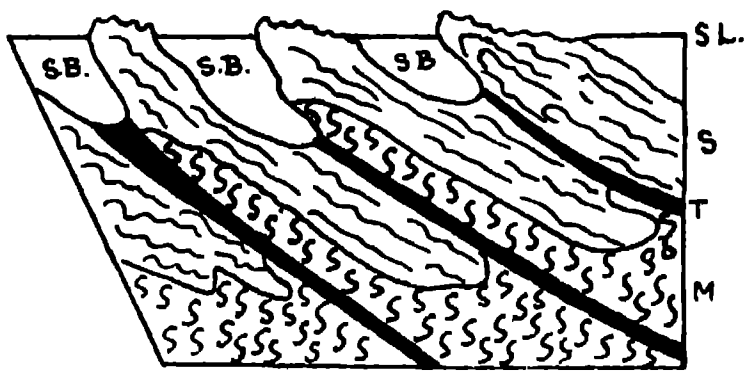


FIG. 2*d*. Mid-Orogen, Eruptive Phase
SB., Sea Basins ; *T*., Thrusts. Further compression and development of deep-seated Thrusts. Ascent of the magma along Thrusts. Submarine Vulcanism

The view advocated here is that though some of these shallow longitudinal seas on drying up may have left sheets of rock-salt, most of these however were by themselves not sufficiently rich or concentrated to give rise to large salt deposits. During certain phases of the orogenesis, however, further compression of the belt led to the development of longitudinal thrust planes some of which extended in depth right to the magma-zone itself (Fig. 2*d*) As a result of intense crustal compression and aided possibly by radio-activity the magma-zone at the base of the geosynclinal belt became heated and mobile and got involved in the general folding of the region The thrust planes served as easy channels of escape and an eruptive activity ensued giving rise to thick dikes and sills and extensive trap formations. Every orogenic zone is replete with such magmatic manifestation nicely illustrated by the ophiolites of the Pennine Alps and by the extensive Tertiary and post-Tertiary volcanic activity in the Circumpacific mountain ranges. It is quite possible that certain deep synclinal basins occupied by the sea had become seats of such fissure eruptions wherein most of the magmatic gases and vapours got absorbed in the sea water. This accession of magmatic emanations to the salts already existing must have violently disturbed the chemical equilibrium of the sea water, whereas the heat of eruption must have raised the temperature of the sea water abnormally high leading to rapid evaporation and even to boiling. This led to the copious precipitation of particularly those salts which were being added from volcanic vents.

Volcanic exhalations include a large number of gases which are given out at different periods according to the phases of the volcanic activity.[6] During the earlier-acid fumarole-phases, sodium chloride and hydrochloric acid are the chief components which are given out abundantly. These are followed by sulphurous acid and at still lower temperatures by hydrogen sulphide when native sulphur gets crystallised. During the later-alkaline fumarole-phases ammonium chloride is the main constituent while carbon dioxide represents nearly the last phase of igneous activity.

In the case of submarine volcanic activity such as we are visualising here the various exhalations are being absorbed by the sea water at different times, and in accordance with the well-known Common-Ion effect, the first salts to get deposited from the sea water are those whose common constituents are being added to the solutions by the magma. Thus during the period when, for instance, volcanic hydrochloric acid is being added to the sea water rock-salt would get precipitated in preference to the less soluble ones

[6] Clarke, F. W., *Data of Geochemistry*, 1924, p. 266.

like calcium sulphate. This principle is actually being commercially utilised in the patented Margueritte process[7] of manufacturing pure common salt from sea water. In the succeeding phases of igneous activity addition of SO_2 or H_2SO_4 would lead to the precipitation of anhydrite (or gypsum) whereas H_2S would deposit sulphur.

In this manner large quantities of different salts would get precipitated from the sea water at different periods and in proportions which are in no way related to the original composition of the sea water.

Under the simple evaporation process as visualised by Ochsenius and others the variation range of temperature is comparatively small allowing only a small number of minerals to be formed under this restricted range.

Under the present theory, however, the sea water would receive heat as well as saline constituents of magmatic origin in a large measure. The sea water would therefore show a much wider range of variation in temperature concentration conditions. This would facilitate crystallisation of a large number of minerals with widely different degrees of solubility and temperatures of crystallization.

A study of minerals actually found in salt deposits shows that besides rock-salt and gypsum a large number of minerals, simple as well as complex, are found which are either hydrous or anhydrous, chlorides, sulphates and often borates and nitrates of sodium, potassium, calcium and magnesium. They show widely varying degrees of solubility at different temperatures in simple or mixed solutions It is, therefore; clear that these minerals will find their appropriate conditions of precipitation much better realised under submarine volcanic conditious than under atmospheric conditions prevailing in shallow evaporating basins

Salt deposits are very commonly associated with effusive rocks rich in alkalies like the rhyolites, ceratophyres, phonolites, etc. It is therefore possible that the volcanic emanations, liquid as well as gaseous, would bring about extensive chemical alterations of these alkaline lava flows and sediments and thereby release abundant materials which would ultimately go into the composition of and add to the quantity of the salts thus formed in the sea. This metasomatic alteration may be so intense that the original identity of the rock may be completely lost. Salt marl which is so constantly seen associated with rock-salt, may owe its peculiar nature to this alteration process.

[7] Thorpe, *Dictionary of Applied Chemistry*, Vol. VI, p 202.

igneous rocks, occurrence of free sulphur, sulphurous gases, boric salts, nitrates, etc.

3. The frequent complicated folding and over-thrusting undergone by salt and associated rock beds, whereby they often show anomalous stratigraphic sequences

4. The extraordinary development of these deposits in certain geological periods like the Permian and the Tertiary which are also the closing period of major Orogeneses.

5. The geographical distribution of rock-salt deposits along definite belts irrespective of latitudes or climatic zones.

6. The parallelism of the salt belt with the belt of the major tectonic mountains, which is also the belt of intense eruptive activity during that orogenic period.

7. The common occurrence of rock-salt and saline lakes in deserts flanking the Tertiary mountain ranges

Summary and Conclusion

From the foregoing treatment it is clear that though some salt deposits may possibly have been formed by simple precipitation in sea or lake waters, brought about by evaporation, lixiviation or by desiccation process, the formation of most of the principal salt deposits of the world is brought about by intra-tectonic submarine igneous activity in geosynclinal basins.

This theory satisfactorily accounts for the various characteristics—chemical, structural, associational and distributional shown by most rock-salt deposits.

If we now extend the scope of this theory, we can also apply it to the formation of certain other saline residues particularly the Borate and the Nitrate deposits, such as those of Chile. which show many characteristics in common with rock-salt.

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STUDIES ON THE CORPUS LUTEUM IN *ENHYDRINA SCHISTOSA* (DAUDIN) AND *HYDROPHIS CYANOCINCTUS* (DAUDIN) OF THE MADRAS COAST

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CONTENTS

Introduction

The corpus luteum of Reptiles has attracted considerable attention and lizards have been the favourite objects of study. In this paper are described, for the first time, the stages of development of the corpus luteum and its degeneration in the viviparous sea-snakes *Enhydrina schistosa* and *Hydrophis cyanocinctus* occurring on the Madras coast.

As early as 1892, Strahl worked out the reconstruction of the ruptured ovarian follicle of *Lacerta agilis* and in 1893 Mingazzini published a paper on "Corpi lutei veri et falsi dei Rettili". Mingazzini working on *Seps chalcides* observes that the follicular epithelium is not discharged at ovulation but undergoes certain changes and takes part in the formation of the corpus luteum. Later on in 1903, Lucien studied the formation of the corpus luteum in the viviparous lizards, *Anguis fragilis* and *Seps chalcides* and published a 'note préliminaire sur les premiéres phases de la formation des corps jaunes chez certaines Reptiles' in which he declared his belief that the corpus luteum is formed by the hypertrophy of the follicular epithelium, unaccompanied by mitotic division, but with invasion of the connective tissue theca Hett's work in 1924 on the corpus luteum in *Lacerta agilis* deals with the structure and development of the follicle and the formation of the corpus luteum after the rupture and escape of the egg. In 1933 Hett published an important review, under the title "Vergleichende Anatomie der Corpora lutea". A comparative study of the corpus luteum in certain oviparous and viviparous lizards by Weekes in 1934 is a more recent contribution. This author gives a description of the histology and development of the corpus luteum in *Amphibolurus muricatus* and *Lygosoma* (*Hinulia*) *quoyi* and a general comparative study of the corpus luteum in the Australian viviparous lizards *Egernia whitei, Lygosoma* (*Hemiergis*) *quadridigitatum, Egernia cunninghami, Lygosoma* (*Liolepisma*) *weekesæ, Lygosoma* (*Liolepisma*) *entrecasteauxi* and the European lizard *Lacerta vivipara*. In the same year Cunningham and Smart published a paper on "The structure and origin of the corpora lutea in lower vertebrates". These authors hold that the formation of the corpus luteum occurs only in viviparous forms and that in oviparous forms the ruptured follicle immediately undergoes degeneration. Boyd's (1940) account of the formation of the corpus luteum in *Hoplodactylus maculatus* is another useful contribution

PART I

ENHYDRINA SCHISTOSA (DAUDIN)

Material and Methods

Six pregnant snakes containing corpora lutea in various stages of development and one non-pregnant specimen containing the largest egg noted and ready for ovulation, form the material for this study. The specimens were collected from November to December, 1940. The snakes were chloroformed and the ovaries quickly dissected out as soon as the animals were dead. For general cytological details Bouin's and Carnoy's fluids were

used and both gave good results. The material was also fixed in a variety of fluids such as Champy, Flemming without acetic, Regaud, corrosive-sublimate and 5% formalin. On account of the thick external coverings of the oocyte and the corpus luteum, fixation and subsequent treatment have to be longer than usual to obtain satisfactory results. Sections 4 to 7 μ in thickness were taken. Iron hæmatoxylin was used after all fixatives and as counterstains Eosin or Van Gieson's. Mallory's triple stain was also used

Both *Enhydrina schistosa* and *Hydrophis cyanocinctus* are viviparous species the young of which are born without egg membranes. Both have a highly specialised allanto-placenta (Kasturirangan, 1941 unpublished) The facts observed indicate that the breeding season of *Enhydrina schistosa* probably extends from November to January. Both the ovaries are functional at the same time. The non-pregnant specimen obtained towards the end of November was found to contain the largest egg examined measuring about 70 mm. in length. Late in the same month ruptured follicles were first observed in the gravid specimens. Between November and December almost all the females examined were pregnant and the ovaries contained corpora lutea in various stages of development. In each ovary, one to three corpora lutea were commonly found. The corpora lutea in the ovary correspond in number to the embryos in each uterus

The Structure of the Unruptured Follicle

The earliest stage of the oocytes obtained shows the round cords of epithelial cells, the egg tubes of ' Pfluger ', growing into the stroma of the ovary where they ramify in complicated anastomoses. The cords enlarge towards the caudal region and become broken up into nests of germinal epithelial cells each of which may be taken to represent a Graffian follicle. At first it is not possible to differentiate the future ova from the cells destined to form the follicular cells. The egg cells become surrounded by a regular layer of small cells representing the follicular epithelium.

The smallest oocytes found in the ovary are situated just beneath the germinal epithelium and are surrounded by a few flattened follicle cells—the primordia of the membrana granulosa. The follicle cells have little cytoplasm but prominent well-developed nuclei containing chromatin reticulum. Slightly larger oocytes are surrounded by a single layer of more or less cubical epithelial cells. The egg now acquires a sheath of undifferentiated connective tissue cells and fibres. At this stage there is no distinction between theca interna and theca externa.

The fairly mature egg, oval in shape, projects slightly from the surface of the ovary. It is invested by the egg membranes—the vitelline membrane

and the zona radiata the latter being thicker and both are closely attached together. In the mature follicle the single layered follicular epithelium has become many layered Two kinds of cells can be distinguished (Ph.M. 1). One set consists of large cells with massive granular cytoplasm each with a prominent nucleus and nucleolus and the chromatin in the form of a few more or less large chromatin granules arranged in a reticulate manner. Intercalated among these large cells are smaller cells with little cytoplasm and with spherical nuclei A nucleolus can be distinguished here also but the chromatin reticulum is not clearly marked. These small cells form an outer layer next to the membrane propria and also extend between the large specialised cells and lie near the zona radiata. The presence of the remarkable large cells—a normal feature of the reptilian follicular epithelium—is interesting as it distinguishes the reptiles from the birds and mammals where the constituent cells are all of one kind. It may be recalled that there is the same difference between the cells of the follicular epithelium in the Raidæ (Samuel, 1943)

The large cells have probably something to do with the nutrition of the egg. Wallace (1904) distinguishes the follicular epithelial cells of *Chimæra* into large " nutritive cells and small indifferent cells " and states " the nutritive cells degenerate and disappear before the maturation of the egg".

Between the follicular epithelium and the connective tissue sheath lies the membrana propria, distinct from the theca folliculi but closely adherent to the follicular epithelium and consisting of a single layer of elongated cells with deeply staining nuclei.

The connective tissue sheath surrounding the follicular epithelium is much more developed, but the distinction between the theca interna and theca externa is not clear though the inner zone contains more of cellular elements (Ph.M. 1). The cells of this zone have spindle-shaped nuclei with their long axes parallel to the follicular epithelium. Minute blood-vessels can be discerned, with some difficulty, in the outer region of the connective tissue sheath.

The fully mature follicle is oval in shape and projects out from the surface of the ovary. The comparatively large size of the egg in the ovary just before ovulation is of interest in connection with the changes which occur after ovulation. The structure of the follicular wall is seen in Fig. 2 and Ph.M. 2. The follicular wall of the egg has undergone considerable stretching as a result of which the membrana propria is no longer seen as a distinct layer. Observations made on the follicular epithelium of the fully developed egg support the statement of Wallace (1904) regarding the

degeneration of the large nutritive cells in *Chimæra*. The large cells have undergone considerable reduction and degeneration. The chromatin of the nuclei clump together accompanied by the complete degeneration and disappearance of the nuclear membrane, cell-cytoplasm and cell-membrane (Ph.M. 2) Leucocytes, probably phagocytic in nature, are of common occurrence. Thus many of the cells break down and disappear and finally in the ruptured follicle there is absolutely no trace of the large cells.

The theca folliculi, surrounding the follicular epithelium, has considerably increased in thickness and is arranged in several layers (Fig 2 and Ph. M. 2). This layer consists of connective tissue cells and fibres, but the parallel arrangement of the fibres is no longer evident as in the previous stage. The cells have oval deeply staining nuclei Although the theca folliculi is greatly developed at this stage the theca interna does not exist as a separate layer. But the elements that would ultimately form it can be distinguished in the vicinity of the follicular epithelium as a narrow layer of connective tissue cells in a loosely arranged network of fibres. In certain regions the theca interna cells and the connective tissue fibres form a more or less definite, if somewhat irregular, layer within the more fibrous outer layer. Immediately outside the theca interna, the connective tissue itself is specially developed into closely approximated fibrous bands with a few cells in the interstices thereof, which later on form the theca externa There is a remarkable increase in the number and size of the blood vessels in this region just before ovulation.

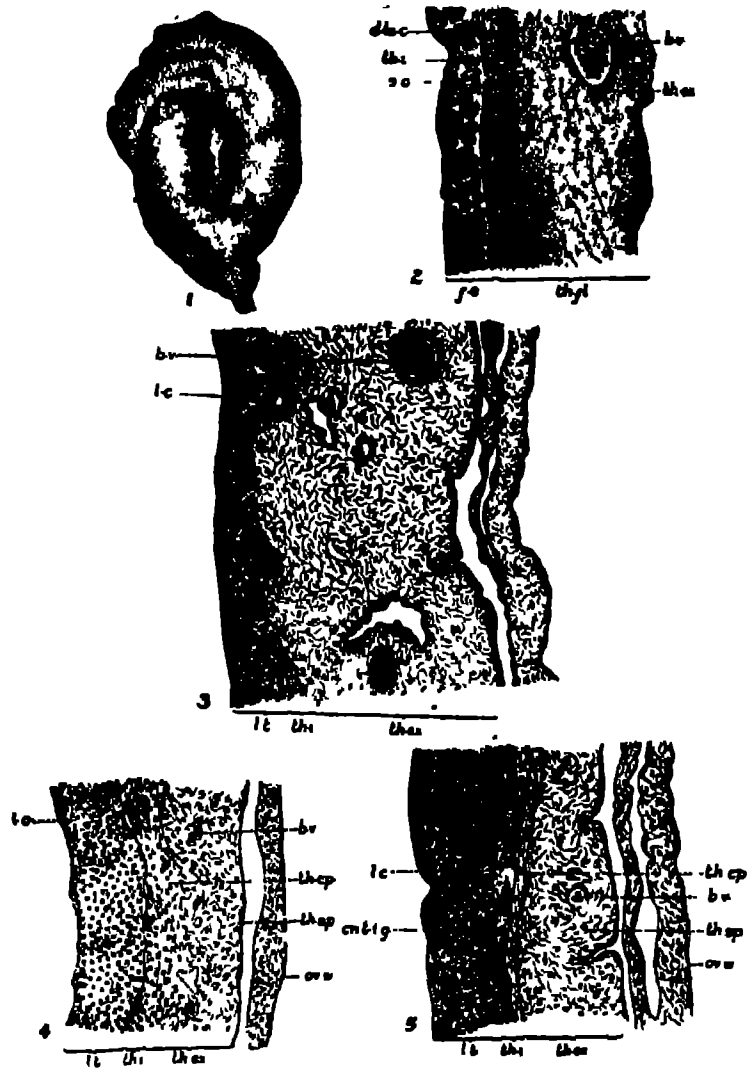
Corpus luteum

Stage I

The uterine eggs corresponding to the corpora lutea in the ovary were in the early blastoderm stage, and all the three eggs present were of the same size and stage of development. It seems that the eggs should have been liberated recently, possibly not more than 3 or 4 days prior to capture of the specimen.

The corpora lutea in the earliest stage are very conspicuous objects by reason of their size (25 mm. in length). They project from the ovary so that the remaining eggs which await a long period of growth and maturation corresponding to the duration of the gestation period appear dwarfs in comparison. All the corpora lutea from this ovary are similar in size and external appearance, and microscopic examination reveals identical cytological features. They are oval bodies situated in the mid-region of the ovary and pinkish white in colour without any trace of the yellow pigment, which is so characteristic of the corpus luteum in later stages. Figure 1 shows the corpus luteum with the ruptured opening of the follicle appearing as

a long slit with its shrunken edges dipping in. Easily visible blood vessels in the sheath tissue vascularize the burst follicle.



FIGS. 1–5.—Fig. 1 The corpus luteum, Stage I, about three times natural size. Fig. 2. T. S of the wall of the fully mature egg showing the follicular epithelium, theca interna and theca externa. ×120. Fig. 3. T S of the corpus luteum, Stage I, to show the hypertrophied follicular epithelial luteal cells, the well-defined theca interna and the theca externa carrying large blood vessels. ×80. Fig. 4. T.S. of the corpus luteum, Stage II, showing the increase in thickness of luteal tissue and the distinction between the theca compacta and theca spongiosa. ×80. Fig. 5. T S. of the corpus luteum, Stage III, showing the change in the three layers, and ingrowth of connective tissue elements into the luteal tissue ×80.

Photomicrograph 3 shows a low power view of a transverse section passing through the ruptured opening of the corpus luteum at this stage. When the follicle bursts and collapses, its walls are brought together so that the central cavity the 'antrum folliculi' is seen as a narrow slit in the centre which communicates to the outside through the opening. The ruptured opening is comparatively large, but gradually becomes smaller and finally closes in the later stages. The central lumen is occupied by a faintly staining coagulum intermingled with a few blood corpuscles and cells. These cells are identical with those forming the follicular epithelium. They are evidently detached from the follicular epithelium, owing to the pressure caused by the rupture and contraction of the wall of the follicle.

A portion of a transverse section of the corpus luteum is illustrated in Ph.M. 4. Owing to contraction, the follicular epithelium lining the lumen becomes folded and it varies from 4 to 10 cells in thickness (Fig. 3 and Ph.M. 4). It consists of large deeply staining luteal cells with characteristic spherical nuclei The cells vary in shape from spherical to polygonal.

The cytoplasm is delicate, finely granular and the cells have definite cell limits. The nucleus stains deeply and possesses a large nucleolus and a well-marked reticulum of deeply staining chromatin granules. It is noteworthy that in the corpus luteum, in striking contrast to the unruptured follicle, we find only one kind of cell—the larger cells having degenerated and absorbed. The corpus luteum at this stage is actually a little smaller than half the size of the ripe egg, consequent on the rupture and escape of the egg—a common phenomenon in certain mammals. The follicular epithelial cells show hypertrophy which is quite evident from the larger size of the cells, in contrast to those of the unruptured follicle. There is an increase in the thickness of the follicular epithelium. This is partly due to the crowding of the cells brought about by the lateral contraction of the follicle and a simultaneous hypertrophy of the cells of the follicular layer. The presence of a few scattered and free blood corpuscles among the differentiating luteal cells is a feature noticed in this early stage. These corpuscles are derived from the vessels of the theca, the walls of which have broken down as a result of the rupture of the follicular wall. This intra-follicular hæmorrhage is a characteristic feature of the higher mammals as described by Marshall (1904) in sheep, etc.

The Theca interna.—The theca folliculi plays a very prominent part in the formation of the early corpus luteum though it undergoes gradual reduction in the later stages. The connective tissue sheath as shown in Fig. 3 and Ph.M. 4 in contrast to its condition in the unruptured follicle has greatly thickened. There is now a clear distinction between the

theca interna and theca externa. Immediately after ovulation the theca interna becomes organised into a layer of connective tissue cells and fibres which lie just below the follicular epithelium (Fig. 3 and Ph.M. 4). The theca interna is comparatively thinner than the fibrous theca externa and follows the wavy contour of the follicular epithelium and has a varying thickness of 2 to 5 cells. The nuclei of the theca interna cells vary in shape and size and a few of them approximate very nearly in size to the nuclei of the follicular epithelial cells from which they can be clearly distinguished. The nuclei are deeply staining and have a distinct nuclear membrane. They contain a large number of intensely staining chromatin granules. The nucleoli are not clearly distinguishable from the chromatin granules in all the cells, as in the case of the follicular epithelial cells. The cytoplasm is faintly vacuolated. Hill and Gatenby (1926) attribute a definite secretory function to the cells of the theca interna which are most active in the earlier stages. A comparison with the unruptured follicle shows a definite increase in the number of the cells. This increase may be due to karyokinetic division though the process has not been observed by me. But Hill and Gatenby (1926) describe and figure a few definite mitotic divisions in the theca interna of the early corpus luteum of *Platypus*. Hett (1924) observes mitotic divisions of the cells of the theca interna in *Lacerta agilis*.

The Theca externa.—The theca externa is more fibrous and very much thicker than the theca interna (Fig 3 and Ph.M. 4). The outer portion of the theca externa is traversed by a number of blood vessels and is distinguished as the theca spongiosa. The inner portion which is more compact, thinner and usually free from any blood vessels is called the theca compacta In the theca externa the connective tissue cells are very rare but is rich in connective tissue fibres and loosely arranged fibro-blastic cells with deeply staining spindle-shaped nuclei. It has already been noticed that the vascularisation of the follicle wall commences immediately prior to ovulation. After rupture there is a greater increase in the number of the large blood vessels in the theca spongiosa. Some of them can be seen penetrating further towards the interior of the follicle. Extravasated blood corpuscles are found not only near the point of rupture of the follicle but also in the theca interna as well as in the theca externa. These blood corpuscles are apparently derived from the large blood vessels of the theca spongiosa the walls of which have broken down.

Stage II

At this stage the blastoderm is well formed and there is a well marked network of blood vessels on the blastoderm. The corpus luteum

corresponding to this stage measures 15 mm. in length. It has undergone marked changes. It is a more or less oval body coloured light yellow.

The central lumen is very much reduced as a result of ingrowths of luteal cells partially filling the cavity which however still communicates with the exterior through a minute opening representing the original rupture.

The Luteal tissue.—The follicular epithelial (luteal) cells now show considerable hypertrophy and present a distinct increase as compared with those of the previous stage. Mitosis has not been noticed by me but has been described in the follicular epithelial cells among the early stages of the formation of the corpus luteum in the Marsupials, *Didelphys aurita* (O'Donoghue, 1916). in *Sheep* (Marshall, 1904) and in the lizard *Amphibolurus muricatus* (Weekes, 1934). The last mentioned author states that follicular epithelial cells divide both mitotically and amitotically but that the mitotic figures are rare. Evidence of a similar multiplication by mitosis has been brought forward by O'Donoghue (1914) in the case of *Perameles obesula* and *Perameles nasuta* None of them mention the phenomenon as very common It is therefore obvious that the increase in the luteal tissue is to be attributed more to the hypertrophy of the already existing cells than to any considerable multiplication as a result of mitosis.

The luteal cells are the largest elements in the corpus luteum, varying in shape from spherical to polygonal cells. The majority of the cells have the characteristic vesicular nuclei, with a large irregular karyosome and a dense network of chromatin granules Some of the nuclei are oval in shape. As the luteal condition is assumed the nucleoli become more and more a distinct feature of the nuclei of the follicular cells. There is a slight variation in the staining capacity among the nuclei of these cells, some of them taking on a lighter stain while others stain deeply with iron hæmatoxylin. The cytoplasm is very finely granular and stains moderately. The perinuclear cytoplasm generally appears to be denser and more deeply staining than the ground cytoplasm surrounding it.

The Theca interna.—Both the theca interna and externa are thinner than in the previous stage and the inner surface of the former has a more even contour (Fig. 4). There is a well-marked difference between the cells of the theca and follicular cells in this stage. The cytoplasm of some of the theca cells is distinctly vacuolated and cell boundaries are not readily distinguishable. The nuclei become more variable in regard to size and shape. Some of them are larger than some of the follicular cell nuclei. The nucleus and the chromatin network are distinct and intensely staining.

Ingrowths resembling little bud-like projections arise from the inner side of the theca interna. It may be stated that the majority of cells come to lie near the follicular epithelium and are most numerous at the base of ingrowths.

The Theca externa.—Though the theca externa is thinner and more fibrous than in the previous stage, the distinction between the theca compacta and theca spongiosa is much more marked than in the earlier stage (Fig. 4). The former is now separate and is formed of parallel layers of closely arranged fibroblastic cells with oval or elongated nuclei of different sizes, varying in thickness from 3 to 5 cells. The large blood vessels are concentrated in the theca spongiosa which is composed of irregularly arranged fibroblastic cells with deeply staining nuclei and connective tissue fibres. There is no appreciable increase in the number of blood vessels.

Stage III

The uterine egg corresponding to the corpus luteum of this stage is approximately in the same stage of development as in the preceding one.

The corpus luteum though of the same shape and size as in the previous stage shows further histological changes. It is almost filled with fully developed luteal cells, except for a small lumen which is seen as a narrow compressed space in the centre in transverse sections passing through the middle. The external opening still persists (Ph.M 5).

The Luteal tissue.—A portion of a transverse section of the entire corpus luteum is shown in Ph.M. 5 Fig. 5 and Ph M. 6 show the structure of the wall of the corpus luteum

The luteal cells with their prominent vesicular nuclei attract attention. They are the largest elements in the corpus luteum and have now attained a relatively large size showing an active glandular appearance (Ph.M. 6). The luteal cells have a distinct wall at this stage and most of them have assumed a spherical shape.

The nuclei are of maximum size, spherical, and apparently in a highly active condition. There is, in each nucleus, a typically large irregular karyosome besides a few smaller nucleoli with all of which a well-marked reticulum studded with deeply staining chromatin granules is connected. The same variation in staining as occurs among the nuclei of the previous stage is noticed in a slightly more marked degree. With the assumption of fully developed luteal condition a regular system of minute vacuoles appears close to the nucleus, in the cytoplasm. They are very few and small at this stage.

The Theca interna.—A close study shows that the connective tissue ingrowths from the theca interna initiated as bud-like projections in the previous stage, are carried further and are in the process of penetration between the luteal cells (Fig. 5, Ph.M. 6). The theca interna cells differ in no essential features from those of the preceding stage but are slightly more vacuolated. Several of them show a clear space between the nucleus and the cell membrane. Inter-cellular spaces begin to appear in the layer. A few scattered blood-corpuscles are present.

The Theca externa—The theca externa is very much of the same thickness as in the previous stage. The nuclei of the fibroblastic cells however appear to be distinctly larger. The fibroblastic cells and fibres of the theca compacta still maintain their parallel arrangement. There is a slight decrease in the vascularisation of the theca and the large blood vessels are confined to the theca spongiosa.

Stage IV

The corpus luteum is slightly reduced in size measuring 12 mm. while the embryo in the uterus has become more advanced measuring approximately 44 mm. in length. The external opening is closed and is marked by a longitudinal depression filled by a mass of yellow substance.

A transverse section of this stage presents a still later phase of development. The central lumen still persists. A plug of luteal tissue projecting slightly to the outside now closes the external opening (Ph.M. 7). Such a protrusion of the luteal cells has been described by many authors in higher mammals, in rabbit by Sobotta (1897), in bat (*Vesperugo noctula*) by Van der Stricht (1912), in pig and cow by Corner (1919) and in *Platypus* by Hill and Gatenby (1926). Corner, in his account of a corresponding protrusion in the pig, says that the hypertrophy of the luteal cells is the cause of the protrusion of a considerable portion of the luteal tissue into the wall of the follicle weakened by the rupture, while in the swine the luteal plug is not of common occurrence because of the distention the whole wall undergoes. Corner further mentions the constant occurrence of the plug of luteal tissue in the corpus luteum of the cow which lasts throughout pregnancy. The protruding plug of luteal cells described in this stage corresponds to that of the above-mentioned authors and to the 'Pfropt' of German authors and that of Van der Stricht (1912) who terms it 'le bouchon épithélial obturator'. But unlike in the cow, this plug becomes soon covered over by the connective tissue sheath in later stages. It may be stated that a similar plug of luteal tissue is not mentioned in the ruptured follicles of reptiles except in *Hoplodactylus* by Boyd (1940). Hett (1924) in his account of the

development and histology of the corpus luteum in *Lacerta agilis* and Weekes (1934), who deals with the corpus luteum in the oviparous *Amphibolurus muricatus* and the viviparous *Lygosoma quoyi* and many other lizards do not make any mention of the formation of this knob-like plug of luteal tissue in the corpus luteum.

The Luteal tissue—A comparison with the previous stage shows (Ph.M. 7) that there is very great increase in the luteal tissue due to the further hypertrophy of the luteal cells. They are large and active looking and their cytoplasm and nuclei resemble in their histological character those of the previous stage. Careful examination reveals that some of the cells have undergone further cytological changes A slight increase in the size of the cell is noticed and most of the cells have spherical shape. The nuclei are slightly reduced in size but a large majority present a characteristic vesicular shape and are more intensely staining. Large numbers of luteal cells show the regular system of spherical vacuoles which are more numerous than in the previous stage. Boyd (1940) in the account of the luteal cells in the corpus luteum of *Hoplodactylus* mentions the appearance of similar small spherical vacuoles containing lipoid globules The observations made on the luteal cells of the present form agree with those of the above author. Hett (1924) mentions in his paper on the corpus luteum of *Lacerta agilis* the appearance of vacuoles in the epithelial cells but he does not describe their nature and structure. Extravasated blood corpuscles, some in the process of degeneration, are found in patches among the luteal cells

The Theca interna—The theca interna is less prominent (Fig. 6) The majority of the nuclei are deeply staining and irregular. There is some decrease in the size of the nuclei of the cells in comparison to the previous stage. These cells form syncytial groups. The septal ingrowths from the theca interna are much more prominent than in earlier stages (Fig. 6 and Ph.M. 7). Extravasated blood corpuscles occur scattered in small patches in the theca interna and along the septal ingrowths as well

The Theca externa.—The theca externa is more reduced in thickness. The line of demarcation between the theca interna and the theca externa continues to be distinct, though not so very clear as in the early stages, while the distinction between the theca compacta and the theca spongiosa is still less definite. Although the septal ingrowths into the luteal tissue take place mainly from the theca interna in the earlier stages, it is clearly seen that strands of connective tissue cells and fibres in close association with the theca interna, are at this period beginning to grow inwards among

the luteal cells. Cell degeneration, evident from the shrunken and collapsed condition of the nuclei of the cells of theca externa are of frequent occurrence at this stage. The large blood-vessels are still confined to the outer region of the theca externa.

Stage V

The corpus luteum as a whole has undergone a further reduction in size and is about 10 mm. in length and the embryo relating to the corpus luteum is 100 mm. in length.

It is a well-developed solid glandular organ densely packed with luteal cells (Ph.M. 8). The central lumen is completely obliterated. The only indication of the initial opening is a shallow groove on the surface The connective tissue sheath has grown over the projecting plug of luteal cells.

The Luteal tissue.—The luteal cells are more closely packed together than in the previous stage (Ph.M. 9). They appear to be smaller and many of them have assumed a more elongated shape although the nuclei appear unchanged. Hence this reduction in size and the elongated shape of the cells may be due to the mutual compression of the cells, caused by the further shrinkage that the corpus luteum has undergone as a whole. The cells are glandular and active. The small spherical vacuoles in the cytoplasm mentioned in the previous stage have become slightly more numerous. The staining capacity of the nuclei is the same in all the cells and all of them take a deep stain.

The Theca.—There is a considerable reduction in the thickness of the sheath tissue (Ph.M. 9) and the distinction between the theca externa and theca interna comprising the connective tissue wall of the follicle cannot be made out either at this stage or hereafter. The reduction in the thickness of the theca may be due to the degeneration of cells occurring in the theca externa in the previous stage and also on account of a superficial penetration of the connective tissue cells of the theca interna between the luteal cells. The invasion of the thecal cells and fibres takes place as radial ingrowths but they do not extend very far into the luteal tissue The blood vessels are still confined to the outer region of the theca.

Stage VI

The corpora lutea described in the three following stages were taken from a female in which the embryos had grown to a larger size measuring approximately 120 mm. The corpora lutea are smaller than in the previous stage. All the three were of equal size measuring 9 mm. in length approximately.

Although the embryos in the uterus do not differ in any marked degree in size and development, the corpora lutea represent three distinct stages in the degeneration of the gland. It is therefore highly probable that once the degeneration processes have started there might be considerable variation in the rate or progress of these changes.

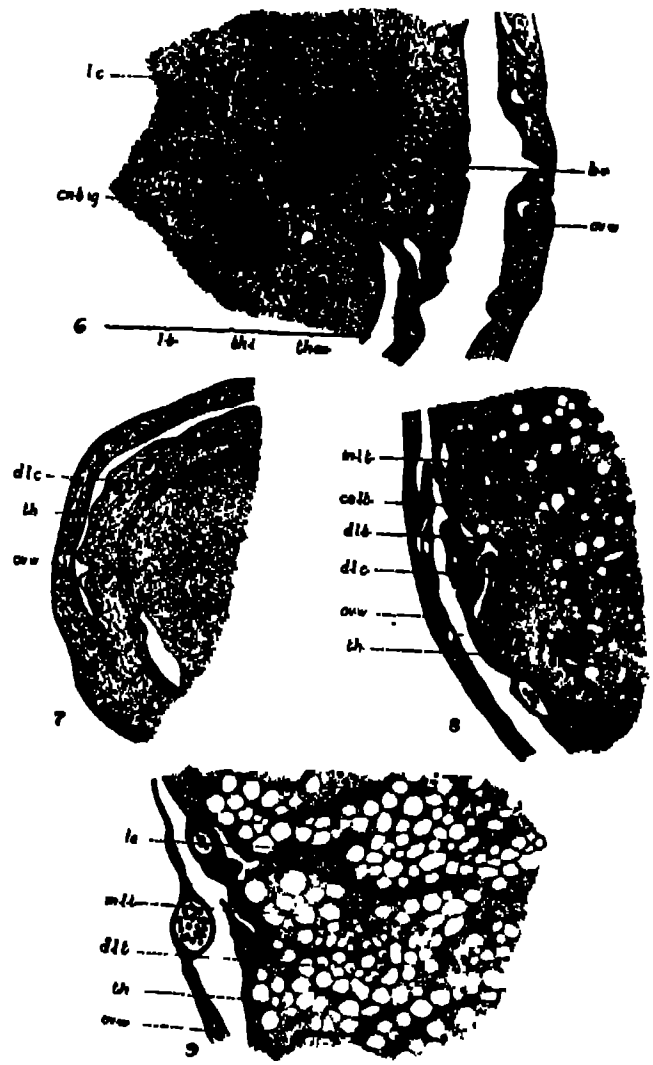
The corpus luteum at this stage shows further reduction. Degeneration has commenced in the luteal tissue. The histological character of many of the luteal cells has altered greatly. Luteal cells showing various stages of degeneration, ranging between fairly normal cells with distinct nucleoli and chromatin network, and shrunken cells with densely staining irregular nuclei and highly vacuolated cytoplasm are seen throughout the whole mass of the luteal tissue (Fig. 7). Weekes (1934) observes vacuolization in the cytoplasm of the luteal cells in the oviparous *Amphibolurus muricatus* and in the viviparous lizard *Lygosoma* (*Hinulia*) *quoyi*. In the latter, towards the latter half of pregnancy the vacuoles become very much enlarged. She infers degeneration from the vacuolated appearance of the cell cytoplasm, the breaking down of the nuclear membrane and the general shrinkage and collapse of all the nuclei. A great majority of the luteal cells have undergone shrinkage and both cell and nuclei are smaller than in the preceding stages. There is a marked increase in the number of intra-cellular vacuoles which are larger, irregular and of varying sizes in contrast to the minute spherical vacuoles of the earlier stages. The cytoplasm of a large majority of cells is completely filled with them. Further evidence of degeneration is seen in the distortion of the nuclei which seems to take place before the loss of the nuclear membrane. Most of them are crenated and shrunken. Clumping of chromatin of these nuclei is evident from the densely staining condition where neither the nucleoli nor chromatin network could be made out. Vacuolar spaces containing pyknotic nuclei and nuclear fragments are not rare.

The connective tissue theca has changed but slightly. It is hardly to be distinguished from the surrounding ovarian stroma. The nuclei of the connective tissue theca cells appear to be smaller than those of the previous stage. The blood vessels, as compared with the early stages, are very much reduced both in number and size, and they are still concentrated in the outer region though a few blood-capillaries are found scattered in the region of the theca interna.

Stage VII

The degeneration of the luteal cells once begun continues until it has spread throughout the corpus luteum. The luteal tissue exhibits a spongy

appearance, owing to the presence of large intracellular vacuoles and numerous spaces in between the cells (Ph.M. 10). Varying phases of



FIGS 6-9.—Fig. 6. T S. of the corpus luteum, Stage IV, showing the great increase in the luteal tissue and prominent ingrowths of the theca interna Fibres from the theca externa are seen to grow along with the theca interna ingrowths. ×80 Fig. 7 T.S. of the corpus luteum, Stage VI, showing the reduction in the thickness of the sheath-tissue and the change in the luteal cells as a result of degeneration. Some have become greatly reduced in size. ×80. Fig. 8. T.S. of the degenerating corpus luteum showing the inter-cellular spaces, the coagulum formed by the degeneration of luteal cells and the reduced theca. ×80. Fig. 9. T.S. of corpus luteum in an advanced stage of degeneration showing the great increase of inter-cellular spaces and the appearance of leucocytes among the degenerating luteal cells. ×80.

regression of luteal tissue can be seen. Many of the luteal cells have undergone further shrinkage and some of them have assumed a more elongated appearance. In the centre of the corpus luteum can be seen some cells with fairly normal structure, containing vesicular nuclei and granular cytoplasm. There is an appreciable increase in the vacuoles in the cytoplasm. Several of them even join together to form one or two big vacuoles which push the nucleus to one side and the whole cytoplasm is seen as a ring round the vacuole.

Further evidence of degeneration can be noted. Darkly staining masses have appeared in the cells which have now lost their cell limits and in which the nuceli have become shrunken and pyknotic. Many of these degenerating cells group themselves together in places. Some of them apparently transform themselves into a structureless coagulum, probably as the result of a further degeneration of these nucleated masses (Fig. 8 and Ph.M. 10) Hill and Gatenby (1926) in *Platypus*, Hett (1924) in *Lacerta agilis*, and Weekes (1934) in *Lygosoma* (*Hinulia*) *quoyi* have observed a corresponding coagulum in the luteal tissue and regarded them as products of cell degeneration. Another evidence of degeneration is seen in the presence of vacuolar spaces containing spherules of varying size and faintly staining capacity which have appeared inside the degenerating luteal cells Similar vacuolar spaces containing colloidal spherules of a finely granular structure in the degenerating corpus luteum are described in *Platypus* by Hill and Gatenby (1926). Vacuolated cells, in which the vacuoles are devoid of formed matter or granular spherules, with irregular shrunken nuclear bodies are very frequent.

The connective tissue sheath is now reduced to such an extent that it is seen as a thin envelope and in certain regions it is only about 2 or 3 cells in thickness (Fig. 8 and Ph M. 10). There is absolutely no distinction between theca interna and theca externa. At the regions where it is in contact with the ovary it is very indistinct and hardly to be distinguished from the surrounding ovarian stroma. The reduced blood vessels are confined to the periphery.

Stage VIII

The corpus luteum has undergone the final regressive changes (Ph.M 11). The cell cytoplasm and nuclei of the luteal cells are in an advanced stage of degeneration. The degeneration is more marked at the periphery than in the central region. A comparison with the earlier stages shows at a glance the widespread degeneration of the luetal cells and the reduction they have undergone. Consequent on the reduction in size and number of cells there is a considerable increase in the number of intercellular spaces

which have become greatly enlarged (Fig. 9 and Ph.M. 12). The vacuoles in the cytoplasm have become very prominent and larger. In the great majority of cells the cytoplasm is very much reduced and all that is left is cell membrane with a thin pellicle of protoplasm enclosing a large single vacuole with the nucleus situated on one side at the periphery. Simultaneous with the degeneration of the luteal tissue large spaces containing leucocytes appear among the regressing luteal tissue (Fig. 9 and Ph.M. 12). They are probably phagocytic in nature and may be concerned with the removal of the degenerated luteal cells in the same way as described by Sandes (1903) in *Dasyurus viverrinus* and by O'Donoghue (1916), in *Didelphys aurita.*

The connective tissue sheath is very thin and reduced which may be another result of degeneration (Fig. 9 and Ph.M. 12). In *Enhydrina* degeneration seems to set in as early as Stage VI, and is well advanced in Stage VIII.

Summary

1. *Enhydrina schistosa*, a sea-snake of the Madras Coast, is viviparous with a highly specialised allanto-placenta. Gravid females were obtained from November to December, 1940, and several ruptured follicles in different stages of the formation of the corpus luteum were studied.

2. The structure of the wall of the mature follicle is described. As in mammals the three layers of the wall of the Graffian follicle concerned in the formation of the corpus luteum, after ovulation are (1) the follicular epithelium, (2) theca interna, (3) theca externa. The histological changes taking place inside the ruptured follicles leading up to the formation of a solid gland and its subsequent degeneration are described in detail.

3. The luteal cells are exclusively formed from the small cells of the follicular epithelium.

4. The theca is greatly thickened after ovulation and shows a clear distinction into theca interna and externa and the latter into theca compacta and spongiosa. There is a gradual reduction of the theca in the later stages accompanied by decrease in vascularity.

5. Ingrowths of cells and fibres from the theca among the luteal tissue take place but these are not accompanied by blood vessels. The fibroblastic cells do not penetrate in between the individual luteal cells.

6. In *Enhydrina schistosa* the active phase of the corpus luteum is comparatively short as degeneration sets in early.

PART II

HYDROPHIS CYANOCINCTUS (DAUDIN)

Hydrophis cyanocinctus is another viviparous sea-snake of the Madras Coast. The material studied was collected from 5 gravid females and one in the post pregnant stage early in 1940. The breeding season probably centres toward March, April and May. The ovary of this species resembles that of *Enhydrina schistosa* in all essential respects, except in the comparatively smaller size of the ova. Two to nine ova attain full size in each ovary simultaneously, three being the usual number of embryos found in each uterus corresponding to the number of corpora lutea in the ovary.

The Structure of the Wall of the Unruptured Follicle

As in *Enhydrina schistosa*, the egg is surrounded by the zona radiata and the vitelline membrane. The wall of the unruptured follicle of snakes has been described in *E. schistosa* and the condition in *H. cyanocinctus* is essentially similar in all the main histological and cytological details. It differs from the former in a few minor details which are given below.

The Follicular epithelium.—The follicular epithelium is a well-defined layer varying from 3 to 4 cells in thickness. It possesses two kinds of cells—large specialised cells, wedged in among which are small cells as in the case of *E. schistosa*. The large follicle cells have prominent vesicular nuclei with a large nucleolus and a network of deeply staining large chromatin granules. The cytoplasm is massive and deeply staining. The majority of large cells are pear-shaped (Fig. 1, Ph.M. 1) and are arranged with their long axes radial to the surface of the egg, their narrow pointed ends being directed towards the surface. The smaller cells possess fairly spherical nuclei, with a nucleolar reticulum. A large number of these small undifferentiated cells lie close to the membrana propria.

The membrana propria considered by most authors as a modified layer of theca folliculi is well marked and distinct from the rest of the theca. It consists of a thin layer of elongated cells with elongated nuclei densely filled with fine chromatin granules (Fig. 1 and Ph.M. 1).

The Theca folliculi.—The theca folliculi is on the whole not so thick as in *E. schistosa*. In *E. schistosa* the theca folliculi is specialised into two layers, an outer fibrous layer, the theca externa and an inner layer the theca interna containing a large number of connective tissue cells. But in *H. cyanocinctus* this differentiation of the theca folliculi into theca externa and theca interna is extremely slight. The rudimentary theca interna, however, is represented by a narrow layer of two or three cells thickness, containing

connective tissue cells with oval or elongated nuclei and fibres running in between them. The cells and fibres are arranged parallel to the follicular epithelium. The theca externa layer surrounding the inner theca is arranged in several layers and consists of connective tissue fibres and occasional fibroblastic cells with elongated nuclei, both of which are disposed parallel to the inner layers. In association with this layer there are a few minute blood vessels.

Corpus luteum

Stage I

The youngest corpus luteum obtained corresponds to an embryo at an early stage of development. It is oval in shape and measures only about 5 mm. in length.

There is a lumen in the centre of the corpus luteum, 'the antrum folliculi' lined by the follicular epithelium, and filled with an honey-combed mass of deeply staining coagulum, intermingled with a few cells. These cells with large spherical nuclei are identical with those forming the follicular epithelium. Evidently they are detached from the follicular epithelium by the rupture and escape of the ovum.

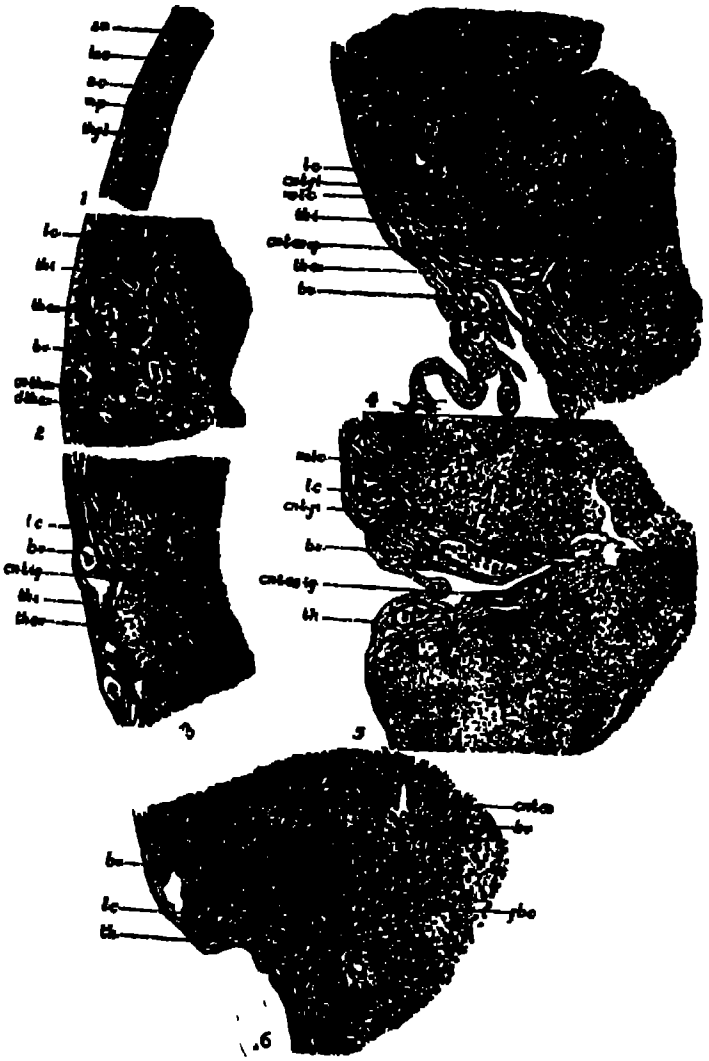
The Luteal tissue (Fig. 2).—As a result of ovulation the follicle as a whole has undergone contraction and the follicular epithelium is thrown into folds. As described in *Enhydrina* the follicular epithelium, in contrast to the unruptured condition, consists of only one kind of cells—the small cells, the large cells having completely disappeared. There is considerable hypertrophy of the follicular epithelium. It is approximately twice the thickness of that of the unruptured follicle and consists of large moderately staining cells of variable shapes, spherical or polygonal with distinct cell boundaries. During the process of transformation of the follicular epithelial cells into luteal cells changes take place that concern both the nucleus and the cytoplasm. The nucleus which hitherto remained small, gradually enlarges and assumes a characteristic vesicular shape, and contains one or two nucleoli. As the luteal condition is assumed nucleoli become more and more a prominent feature of the nuclei. A slight variation in the staining capacity of these cells is noticed. Some of the cells take a light stain while others are intensely stained. The cytoplasm stains moderately and is finely granular, the granules being diffusely scattered throughout the cytoplasm and they show up fairly with iron alum hæmatoxylin.

The Theca (Fig. 2).—Coincident with these processes in the follicular epithelium, certain histological changes take place in the theca folliculi. The membrana propria is no longer seen as a distinct layer After the rupture

of the follicle, the theca is much more thickened which may be due to shrinkage of the follicle. In comparison with the remarkable thickening and differentiation of the theca folliculi into a distinct theca interna rich in connective tissue cells and a fibrous theca externa in *E. schistosa*, the distinction here is comparatively slight.

The Theca interna.—The theca interna is seen as a narrow layer close round the follicular epithelium and follows the wavy contour of the epithelial layer. This layer consists of connective tissue cells and fibres running in between them (Fig. 2). The theca interna cells are totally unlike the luteal cells of the follicular epithelium and can be readily distinguished from them. The cells of the theca interna at no time approximate to the luteal cells in size. But their average size is larger than in the unruptured condition. Their bounding cell membranes are usually indistinct, their nuclei vary from oval to elongate and they stain rather deeply and show a nucleolar body and a reticulum of minute chromatin granules. The cytoplasm stains moderately and is frequently vacuolated. This fibrocellular layer varies in thickness and the cells and fibres run parallel with the surface of the follicular epithelium. At this stage there is no sign of any inward growth of the connective tissue from the theca. It is during this period that the theca interna reaches its maximum development. As the corpus luteum advances in development the differentiation of the theca folliculi into theca externa and interna becomes practically indistinguishable and the latter is only represented by an indistinct layer of small oval nucleated cells and fibres, closely resembling the theca externa lying close to the follicular epithelium.

The Theca externa (Fig. 2).—The theca externa surrounding the follicle has increased in thickness after the rupture and is many times thicker than the theca interna. In *E. schistosa* the theca externa is differentiated into two layers, an inner compact layer called the theca compacta and an outer irregular one containing the large blood-vessels, the theca spongiosa. In *H. cyanocinctus* this differentiation of the theca externa into theca compacta and theca spongiosa is extremely slight. The outer region of the theca carries a few small blood vessels. In the connective tissue substance of the theca externa nuclear degeneration could be clearly noticed. Some of the fibroblastic cells of the theca externa can be seen surrounding the walls of the blood vessels. Vascularisation is very slight in earlier stages but gradually increases in later stages accompanied by a greater development of the connective tissue sheath.



FIGS. 1–6.—Fig. 1 T.S. of the unruptured follicle showing the follicular wall. ×120. Fig. 2. T.S. of the corpus luteum, Stage I, showing the three layers. ×80 Fig 3. T.S of the corpus luteum, Stage II, showing the increase in the luteal tissue and reduction in the theca and the ingrowth of connective tissue ×80. Fig 4. T S of the corpus luteum, Stage III, showing the great increase in the luteal tissue and the ingrowth of connective tissue septa and the fibres cutting the luteal cells into 'nests'. ×80. Fig. 5. T S. of the corpus luteum, Stage IV, showing the prominent ingrowth of the connective tissue septa and the invasion of blood vessels along with it. ×80. Fig. 6. T S. of the corpus luteum, Stage V, showing the extensive development of the septum which carries the blood vessels and the penetration of fibroblastic cells among the individual luteal cells. ×80.

Stage II

The corpus luteum is more or less spherical and is about 6 mm. × 5 mm. in diameter. The embryo relating to the corpus luteum described in this stage is about 122 mm. in length.

This stage shows a further advance. The whole gland has slightly increased in size. There is a large oval lumen in contrast to the compressed elongated lumen observed in *E. schistosa* (Ph.M. 2). Now there is no trace of the deeply staining coagulum in the lumen. The opening of ruptured spot is occupied by a mass of luteal cells which is more or less exposed and partially devoid of connective tissue covering. The rapid enlargement of the luteal cells in a limited space must have caused the protrusion of the cells into the opening left by the escape of the egg. A corresponding protrusion of the luteal tissue has been described in *E. schistosa* but the luteal plug is smaller in this case because of the enlargement, the follicle undergoes as a whole. In *H cyanocinctus* the ruptured opening is almost immediately closed by a plug of luteal cells and the coming together of the connective tissue wall.

The Luteal tissue.—The luteal tissue is more massive and the thickness of the epithelial wall of the corpus luteum as shown in a transverse section has increased appreciably (Fig. 3 and Ph.M. 2). It is almost thrice the thickness of that of the previous stage, the increase being due mainly to the simple hypertrophy of the individual cells composing it. They vary in shape from spherical to polygonal and have distinct cell limits as in the previous case. The cytoplasm generally appears in reticulate form and stains moderately. The nucleus has become slightly larger and typically vesicular and contains a large karyosome, usually two nucleolar bodies and a distinct chromatin reticulum. The variation in the staining capacity of the nuclei is still more prominent.

The Theca (Fig. 3 and Ph.M. 2).—The theca has undergone a marked stretching and consequent reduction in thickness due to the enlargement of the luteal cells. The line of demarcation between the theca interna and theca externa is less distinct but there is slight increase in the number of blood vessels, which are apparently confined to the theca externa. From the theca interna connective tissue fibres and cells are in the process of invading the luteal tissue (Fig. 3).

Stage III

The corpus luteum has an oval shape and measures 10 mm. in length. The embryo in the uterus corresponding to the corpus luteum studied is 142 mm. in length.

Photomicrograph 3 shows that the central lumen is further reduced by the growth of the luteal cells towards the centre. The connective tissue has covered over the plug of luteal cells.

The Luteal tissue (Fig. 4 and Ph.M. 3)—There is an increase in thickness of the luteal tissue due to mere hypertrophy of the cells as no mitosis has been observed. This leads to the gradual filling up of the central lumen. The luteal cells have attained a larger size with relatively large vesicular or oval nuclei. The nuclei, rich in chromatin granules, stain more deeply than in the previous stage but the variation in the staining capacity is less. The cytoplasm of a few cells shows a regular system of minute spherical vacuoles round the nuceli.

The Theca.—The distinction between the theca interna and externa is still less definite. At the periphery of the corpus luteum the luteal tissue is cut into 'nests' of luteal cells by the ingrowth of connective tissue fibres from the theca interna (Fig 4 and Ph.M. 3). Although the theca interna is very much reduced and hardly distinguishable from the theca externa, it can be seen pushing inwards among the luteal cells as radial ingrowths. This reduction of the theca interna may be due to the ingrowths of connective tissue cells and fibres from this layer which have become much pronounced in this stage. A few small blood capillaries are found in this layer.

The most remarkable characteristic of this stage is the increase in number of blood vessels of considerable size in the theca externa (Ph.M. 3). They are found to be far more abundant than in any of the previous stages. The large blood vessels are concentrated at the periphery of the theca externa where they are surrounded by strands of fibroblastic cells of the theca externa. Although the radial ingrowths into the luteal tissue are chiefly from the reduced theca interna, strands of fibroblastic cells from the theca externa in close association with those of the internal layer are at this stage beginning to grow inwards.

Stage IV

The corpus luteum is about the same size as in the previous stage but the embryo has reached a length of 190 mm.

The Luteal tissue.—A transverse section (Ph.M. 4) of this stage shows a still greater advance. The luteal cells are slightly larger than in the previous stage but the cytological character of most of the cells remains practically unchanged There is a slight increase in number of the minute spherical vacuoles in the cytoplasm of some of the cells. The luteal cells

have grown enormously and almost obliterate the lumen which can be observed only as a narrow slit in a few sections passing through the centre of the corpus luteum.

The Theca.—The theca interna has undergone further attenuation and is hardly distinguishable from the theca externa. There is a corresponding thinning of the sheath tissue. The connective tissue fibres have grown inwards among the luteal cells from all over the inner surface of the theca interna and as a result, the number of 'nests' of luteal cells at the periphery have become very numerous (Fig. 5 and Ph.M. 4).

The corpus luteum exhibits a radially divided appearance which is emphasised by the large strands of fibroblastic radial septal ingrowths from the theca (Fig. 5 and Ph.M. 4). An important feature of the corpus luteum at this stage as illustrated in Ph.M. 4 and Fig 5 is that the large blood vessels in association with the fibroblastic septal ingrowths of the theca externa, which were hitherto confined to the periphery of the theca externa have now grown into the luteal tissue. The connective tissue fibres alone penetrate from the theca interna between the luteal cells, as mentioned above, and form a network among them (Fig. 5 and Ph M. 4).

Stage V

In the next stage of development the corpus luteum has increased in size and is approximately 12 mm. in length while the embryo relating to this stage is 195 mm.

The corpus luteum has become solid throughout and has attained the height of its histological differentiation (Ph.M. 5).

The Luteal tissue.—The luteal cells are glandular and active and are larger than those of the preceding stages (Ph.M. 6). The cell boundaries are distinct. The spherical cytoplasmic vacuoles have greatly increased in number and in a few cells the whole cytoplasm is filled with them. The nuclei are large and prominent and contain a large irregular karyosome and one or two smaller nucleoli with all of which a network of deeply staining chromatin granules is connected. When the nucleus has reached its maximum growth it presents a striking vesicular appearance (Ph.M. 6). Some of the nuclei take a deeper stain than others but this variation in the staining capacity is not so marked as in some of the earlier stages. No nuclear degeneration has been noticed in any of these cells. There is a greater increase of luteal tissue as compared with the earlier stages. Since there is no evidence of cell division either mitotically or amitotically we come to the conclusion that the increase has been mainly brought about by simple

hypertrophy of the luteal cells. If mitotic division occurs at all it must be limited for a short time to the early stages, just after ovulation.

The Theca.—Even though the hypertrophy of the luteal cells is the main factor in bringing about the solidification of the corpus luteum in the later stages, the ingrowth of connective tissue from the theca together with the blood vessels also facilitates the process.

The septa of fibroblastic cells from the theca have penetrated throughout the luteal tissue carrying blood vessels (Fig. 6 and Ph.M. 5). Consequent on the increase of connective tissue ingrowth among the luteal cells, the outer sheath has undergone reduction in thickness and is seen as a thin envelope, 2 to 3 cells thick in certain regions. The connective tissue fibroblastic cells and fibres grow in all directions so that the luteal cells become surrounded by an anastomosis of these elements (Fig. 6 and Ph.M. 6). Thus the connective tissue elements of the theca contribute to the formation of the supporting framework for the luteal cells and the the blood vessels.

Stage VI

The corpus luteum is kidney-shaped and is considerably smaller than in the preceding stage. It measured 6 mm. Since no embryo was obtained it appears that the uterus is in the post-pregnant stage Though very small in size the corpus luteum was readily distinguishable from the white ova by its yellow colour A careful study reveals signs of degeneration in some of the luteal cells. Those towards the centre have undergone shrinkage and are smaller than the similarly situated cells described in Stage V. Cells with deeply staining crenate nuclei are occasionally seen, their presence indicating commencement of degeneration in luteal cells There is an appreciable increase in the size of the vacuoles in the cytoplasm which have now become irregular. Another evidence of degeneration is seen in the appearance of inter-cellular spaces in the middle of the luteal tissue. Faintly staining masses of coagula could be seen here and there These are all indications that the degeneration processes are in full operation.

The connective tissue theca does not appear to have changed in any appreciable manner But in the meanwhile the inter-epithelial connective tissue has become more finely distributed

Summary

1. The formation of the corpus luteum in *Hydrophis cyanocinctus*, another viviparous snake with allanto-placenta, is fundamentally similar to

that of *Enhydrina schistosa* in all essential features. The luteal cells are derived by hypertrophy from the small cells of the follicular epithelium.

2. There is a very slight distinction between the theca interna and theca externa. The fibroblastic cells and the fibres of the theca show greater development with a simultaneous increase in the later stages of the number of blood vessels accompanied by extensive ingrowths of connective tissue septa carrying blood vessels into luteal tissue together with penetration of fibroblastic cells in between the luteal cells.

3. The corpus luteum in the present form has a long intra-ovarian existence and signs of degeneration of luteal cells are noticed only after the birth of the young.

Discussion

Despite the difference in their zoological position the reptiles and mammals show considerable agreement in the development and structure of the corpus luteum. In reptiles as in the mammals, the three layers of the wall of the ruptured follicle, the follicular epithelium, theca interna and theca externa are concerned in its formation. The corpus luteum of the viviparous sea-snakes offers marked advantages for the observation of the changes undergone by the different layers. The corpus luteum in the fully developed condition is a solid glandular organ formed by the hypertrophy of the small cells of the follicular epithelium reinforced with the connective tissue from the theca.

The histological structure and appearance of the corpus luteum in *Enhydrina schistosa* and *Hydrophis cyanocinctus* are fundamentally similar. The corpus luteum in *Enhydrina schistosa* is the largest yet described among the reptiles. In *H. cyanocinctus* it is smaller in the early stages but gradually enlarges though in no stage does it exceed the size of the corpus luteum in *E. schistosa*.

Luteal cells—The luteal cells are derived exclusively from the follicular epithelium in both the forms, in striking contrast to the condition in *Rhinobatus granulatus* (Samuel, 1943). Mitotic divisions have been recorded in the follicular epithelial cells in the early stages of the formation of the corpus luteum in certain marsupials (O'Donoghue, 1914 and 1916), in sheep (Marshall, 1904) and in the lizard *Amphibolurus muricatus* (Weekes, 1934). Hett (1924) who worked on the corpus luteum of *Lacerta agilis* and Boyd (1940) who studied the gland in *Hoplodactylus maculatus* have however, failed to observe mitotic divisions among the luteal cells, in the forms studied and it is obvious, that if there is any mitosis at all it cannot be very

extensive. The major portion of the increase in the luteal tissue must therefore be brought about by simple hypertrophy of the cells.

Our knowledge of the cytological structure and secretory activity of the luteal cells is very limited. There seem to be two phases in the deposition of fat in the corpus luteum of snakes, *viz.*, a lipoidal secretion and a later formation of fat as in the higher mammals. An important feature of the Eutherian luteal cells is the presence of secretory globules. In material fixed in Bouin and corrossive acetic these are present after the usual histological procedure as chains or groups of regular, empty vacuoles. It was not possible for me to study the secretory history of the luteal cells due to scarcity of material. However, the presence of small spherical vacuoles of equal size and similar shape suggests that globules secreted in the fully mature, active corpus luteum are of a lipoid nature. They seem to represent the lipoid secretions of the higher vertebrates. These lipoid globules are readily removed in the non-osmic fixatives used and in the subsequent procedure, and the position of these globules is marked by a regular system of minute spherical vacuoles.

The degree of development of these spherical vacuoles in *H. cyanocinctus* is greater than that in *E. schistosa.* This seems to be correlated with the longer functional activity of the corpus luteum in *H. cyanocinctus* in contrast to the lesser development and apparently shorter functional life of the luteal cells in *E. schistosa.* These vacuoles are extremely small and inconspicuous in comparison with similar vacuoles observed in *Rhinobatus.* In view of the constant occurrence of such globules in the luteal cells of mammals, their presence in the snakes is of interest.

The later appearance of large irregular vacuoles of various sizes and shapes, lacking the uniformity present in the early stages, may be due to the deposition of fat as a sign of fatty degeneration of the cells. This is further supported by the fact that the appearance of these large irregular vacuoles is accompanied by degenerative changes in the nuclei.

Boyd (1940) describes in the luteal cells of *Hoplodactylus maculatus,* the presence of minute vacuoles about the nucleus, occupied by a lipoid substance which blackens with osmium tetroxide. In later stages of the corpus luteum, Boyd further states that " the luteal tissue is very vacuolated, both the size and number of the vacuoles having increased, giving the tissue the appearance of a network ". Observations made on the luteal cells of the present forms agree with those of the above author. Hett (1924) records the appearance of vacuoles in the luteal cells of *Lacerta agilis,* both in early and late stages, but he does not express any opinion regarding the

nature of the secretory products of the luteal cells. Weekes (1934) mentions the appearance of large vacuoles in the cytoplasm of luteal cells in the later stages both in *Amphibolurus muricatus* and *Lygosoma* (*Hinulia*) *quoyi* According to this author, these vacuoles become very pronounced at the time of the birth of the young In her opinion the appearance of large vacuoles in the cytoplasm together with the breaking down of the nuclear membrane and general shrinkage and collapse of the nuclei is a sign of regression of the tissue. Weekes, however, does not mention the presence of a regular system of minute spherical vacuoles This may be attributed to the minute size of these vacuoles

The Theca.—The structure and subsequent behaviour of the theca folliculi vary greatly in the two species studied. In *E. schistosa* immediately after ovulation, the theca is greatly thickened and differentiated into theca interna and theca externa. The theca externa further shows distinction into theca compacta and theca spongiosa. In this respect *E. schistosa* approaches the condition described in mammals and agrees with lizards such as *Amphibolurus muricatus*, *Lygosoma* (*Hinulia*) *quoyi* (Weekes, 1934), and *Lacerta agilis* (Hett, 1924). This distinction between the theca interna and theca externa is fairly well marked and constant up to a late stage.

Even the little distinction that exists between the theca externa and theca interna in the early stages in *H. cyanocinctus* is lost in the later stages. Another point of difference is that the connective tissue fibres and fibroblastic cells are very compactly arranged in the theca in *H. cyanocinctus* while they are loosely scattered in *E. schistosa* These variations seem to be pretty common Boyd (1940) for instance has called attention to the fact that distinction between the theca interna and theca externa does not occur in *Hoplodactylus*. O'Donoghue (1914) observes great variation in the degree of development of the theca interna in Marsupials. In *Phascolomys*, the theca interna is well developed, while it is extremely rudimentary in *Dasyurus*.

Variations occur in regard to the ingrowth of the theca into the luteal tissue, in the two species studied. In *E. schistosa* in the early stages, connective tissue septal ingrowths of cells and fibres unassociated with blood vessels takes place from the theca interna into the luteal tissue and later strands of connective tissue cells and fibres from the theca externa also join in this invasion. The connective tissue fibres and cells in *E. schistosa* do not form an anastomosis of fusiform cells surrounding the luteal cells as in *H. cyanocinctus*. In *H. cyanocinctus* there is an extensive ingrowth of connective tissue septa carrying large blood vessels. The fibres and fibroblastic cells invade among the luteal cells and nearly all the cells become surrounded by a network of fibroblastic cells and fibres.

Weekes (1934) distinguishes three kinds of behaviour of the theca in the lizards. In *L. quoyi, L. quadridigitatum* and *E. whitei* there is no invasion of theca interna fibroblastic cells in amongst the individual luteal cells. In *L. Weekesæ* and *L. entrecasteauxi,* there is ingrowth of strands of fibroblasts in association with blood vessels, but they do not penetrate between the individual luteal cells and thirdly as seen in *L. vivipara,* the spetal growth carrying the blood vessels and a penetration of fibroblastic cells among the luteal cells takes place.

Boyd states that *Lacerta vivipara* and *Hoplodactylus* (Boyd, 1940) "are the only ones so far in reptiles where fibroblasts penetrate between the individual lutein cells as well as form septa", but that "In *Lacerta vivipara,* however, blood-vessels grow in with the connective tissue". It is noteworthy that in *Hydrophis cyanocinctus* we find both the penetration of the fibroblastic cells among the luteal cells and the ingrowth of the blood vessels in association with fibroblastic septa. Hence the similarity between the *L. vivipara* and *H. cyanocinctus* is more complete. In regard to the ingrowth of connective tissue theca, *Enhydrina schistosa* shows similarity to *Lygosoma quoyi,* in which there is only a superficial ingrowth of fibroblastic cells among the luteal cells, and no blood vessels are found among them.

Another feature of interest is the difference in the time of vascularisation of the corpus luteum in the two forms studied. In *E. schistosa,* soon after ovulation, there is a great development of blood vessels in the greatly thickened theca externa which becomes gradually reduced in later stages; On the other hand in *H. cyanocinctus* the theca is poorly vascularised in the early stages but vascularisation increases later accompanied by a greater development of the theca. It has been suggested by Deanesly (1930 *b*) for the mammals that this variation in the development of the theca and blood vessels can be attributed to the difference in size of the ripe follicle.

Although there is fundamental similarity in the structure of the corpus luteum in both the forms, the duration of functional activity of the gland in relation to the development of the embryo in the uterus shows considerable difference in the two forms studied. In *E. schistosa* the corpus luteum attains its full growth when the embryo is about 44 mm. and becomes solid by the time the embryo attains a length of 100 mm. Regression sets in shortly after and is well advanced when the embryo is about 120 mm. Degenerative changes once begun, advance rapidly and the final stages of degeneration are in evidence even when embryos are of the same length. In *H. cyanocinctus* the corpus luteum is at its height of histological development when the embryo is 195 mm. and evident signs of degeneration are observed only after the birth of the young.

In the viviparous lizards, Weekes (1934) states that the corpus luteum has an intra-ovarian existence throughout the gestation period and disappears only after the birth of the young. According to Boyd (1940), in *Hoplodactylus* the corpus luteum remains in the ovary even after the birth of the young though degeneration has set in. With regard to the long intra-uterine existence of the corpus luteum *H. cyanocinctus* resembles the viviparous lizards while *E. schistosa* shows resemblance to the monotremes where 'regression appears to set in remarkably early' (Hill and Gatenby, 1926). Apparently the functional activity of the corpus luteum is most important only in the early stages of development of the embryo as otherwise it is difficult to explain its later behaviour in the various animals studied.

A review of our present knowledge of the corpus luteum in reptiles in general, will be given in a paper on the corpus luteum of another viviparous snake, *Cerberus rhyncops*, under preparation.

Acknowledgments

I wish to express my grateful thanks to Prof. R. Gopala Aiyar, Director, University Zoological Research Laboratory, under whose guidance this work was carried out. I am also indebted to the University of Madras for the award of a research studentship.

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EXPLANATION OF PHOTOMICROGRAPHS

PART I

PLATE IV

Photomicrograph 1. T.S. of a fairly mature egg showing the two kinds of cells of the follicular epithelium.

„ 2. T.S. of the wall of the fully matured follicle showing the degeneration of large cells and the thickened theca

„ 3. T.S. of the corpus luteum, Stage I, showing the rupture.

„ 4. T.S of the corpus luteum, Stage I, showing the three layers of the wall follicular epithelium, theca interna and theca externa with the large blood vessels.

Photo-micrograph 5 T S of the corpus luteum Stage III, showing the ruptured opening

,, 6 T S of the corpus luteum, Stage III, showing the wall and ingrowth of theca interna elements into the luteal tissue

PLATE V

Photo-micrograph 7 T S of the corpus luteum, Stage IV, showing the reduced central lumen and the increase in the luteal tissue

,, 8. T S of the solid corpus luteum, Stage V

,, 9 T S of the corpus luteum, Stage V, showing a portion to illustrate the closely packed luteal cells

,, 10 T S of the degenerating corpus luteum, Stage VII, showing the coagulum in the luteal tissue, inter-cellular spaces in the luteal tissue, and the reduced theca

,, 11 T S of the degenerating corpus luteum, Stage VIII, presenting a highly degenerate stage of luteal cells

,, 12 T S of the corpus luteum, Stage VIII a portion magnified to show the large number of intercellular spaces and the appearance of leucocytes among the degenerating luteal cells

PART II

PLATE VI

Photo-micrograph 1 T S of the unruptured follicle showing the pear-shaped large cells, and the small cells of the follicular epithelium

,, 2 T S of the corpus luteum, Stage II

,, 3 T S of the corpus luteum, Stage III, showing the ingrowth of connctivee tissue septa and the increase in luteal tissue

,, 4 T S of the corpus luteum, Stage IV showing the prominent septa and the invasion of blood vessels along with them and the 'nests' of luteal cells.

,, 5 T S of the corpus luteum, Stage V.

,, 6 The luteal cells of Stage V magnified to show the large size of the cells and the network of fibroblastic cells surrounding the luteal cells

KEY TO LETTERING

b v.	Blood vessels.	*le*	Leucocytes
cn.t.ig.	Connective tissue ingrowth	*m.p*	Membrana propria
cn.t.se ig	Connective tissue septal ingrowth.	*ne.l.c*	Nests of luteal cell
co.l.t	Coagulum in luteal tissue	*ov w*	Ovarian wall
d.l.c.	Degenerating luteal cell.	*pl l t.*	Plug of luteal tissue.
d l t.	Degenerating luteal tissue	*ro*	Ruptured opening.
d.la.c.	Degenerating large cell of the follicular epithelium	*s c*	Small cell of the follicular epithelium.
f.e.	Follicular epithelium.		Theca
b.c.	Fibroblastic cells	*th cp*	Theca compacta.
in.l.t	Intercellular species in the luteal tissue	*th ex*	Theca externa
l.c.	Luteal cell	*th fl*	Theca folliculi.
l t.	Luteal tissue.	*th i*	Theca interna
la.c.	Larger cell of the follicular epithelium.	*th.sp.*	Theca spongiosa
		z r.	Zona radiata.

N.B.—All figures have been reduced to half the dimensions given.

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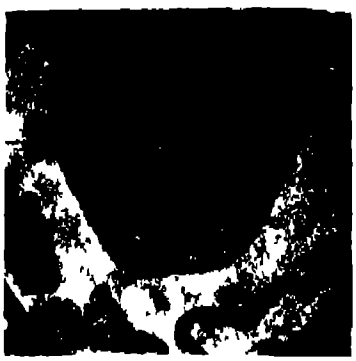

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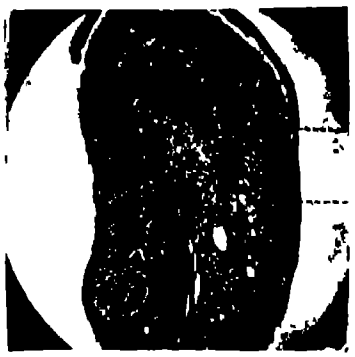

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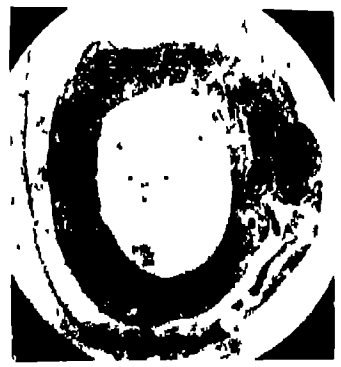

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GAMETOGENESIS AND EMBRYOGENY IN A FEW MEMBERS OF THE ARALIACEÆ*

By D. M. Gopinath

Received September 7, 1944
(Communicated by Prof. L. Narayan Rao, Ph D, F.A.Sc)

Introduction

Literature on the developmental morphology of the family Araliaceæ dates as far back as 1902, when Ducamp first published his "Recherches sur l'Embryogenie des Araliacees." Further studies on the embryology of some members of the Araliaceæ were taken up by Prankard (1914), Pigott (1914, 1927), Cammerloher (1910), etc The present work was undertaken with a view to throw more knowledge on the subject regarding the gametogenesis and embryogeny in some members of the family. The following plants were taken up for study:

(i) *Panax fruticosa* Linn
(ii) *Polyscias pinnata* Linn
(iii) *Hedera australiana* F. Muell
(iv) *Heptapleuram venulosum* Seem
(v) *Brassaia actinophylla* Endl.

With the exception of *Heptapleuram venulosum* Seem which is a shrubby climber occurring in many cases as epiphytes (Thirumalachar, Swamy and K. B. A. Khan, 1942) the other plants taken up for study were obtained from the Government Botanical Gardens, Bangalore *Heptapleuram venulosum* was collected round-about Bangalore.

Materials for cytological work were collected and fixed in various fixatives by following the usual process. Sections from 6–18 μ thickness were cut and stained. Heidenhain's iron-alum hæmotoxylin with eosin in clove oil as counter-stain was mostly used. A hot mordant in iron-alum at 50° C. was tried in a few cases and the results were especially good.

Microsporogenesis

Microsporogenesis was studied in *Panax fruticosa*, *Hedera australiana* and *Brassia actinophylla*. There are four to six stamens in *P. fruticosa*, five in *H. australiana* and six to eighteen in *B. actinophylla* corresponding to the petal lobes. The anther shows a four locular nature in cross-section

* Thesis approved for the Master's Degree examination, 1941, Mysore University.

(Fig. 43). The archesporium consists of two to three layers of cells and these divide to form outer parietal layers and inner sporogenous layers The parietal layer divides further and forms three to four layers of cells The outermost of these layers forms the endothecial layer The middle layers vary in number In *P fruticosa* and *H australiana* only a single wall layer is present whereas in *B actinophylla* there are two layers. The sporogenous cells divide further and give rise to a number of microspore mother cells. The tapetal layer is closely adpressed to the microspore mother cells The cells of the tapetum are large in size, binucleate in *P fruticosa* and *B. actinophylla* In *H australiana* they are invariably uninucleate

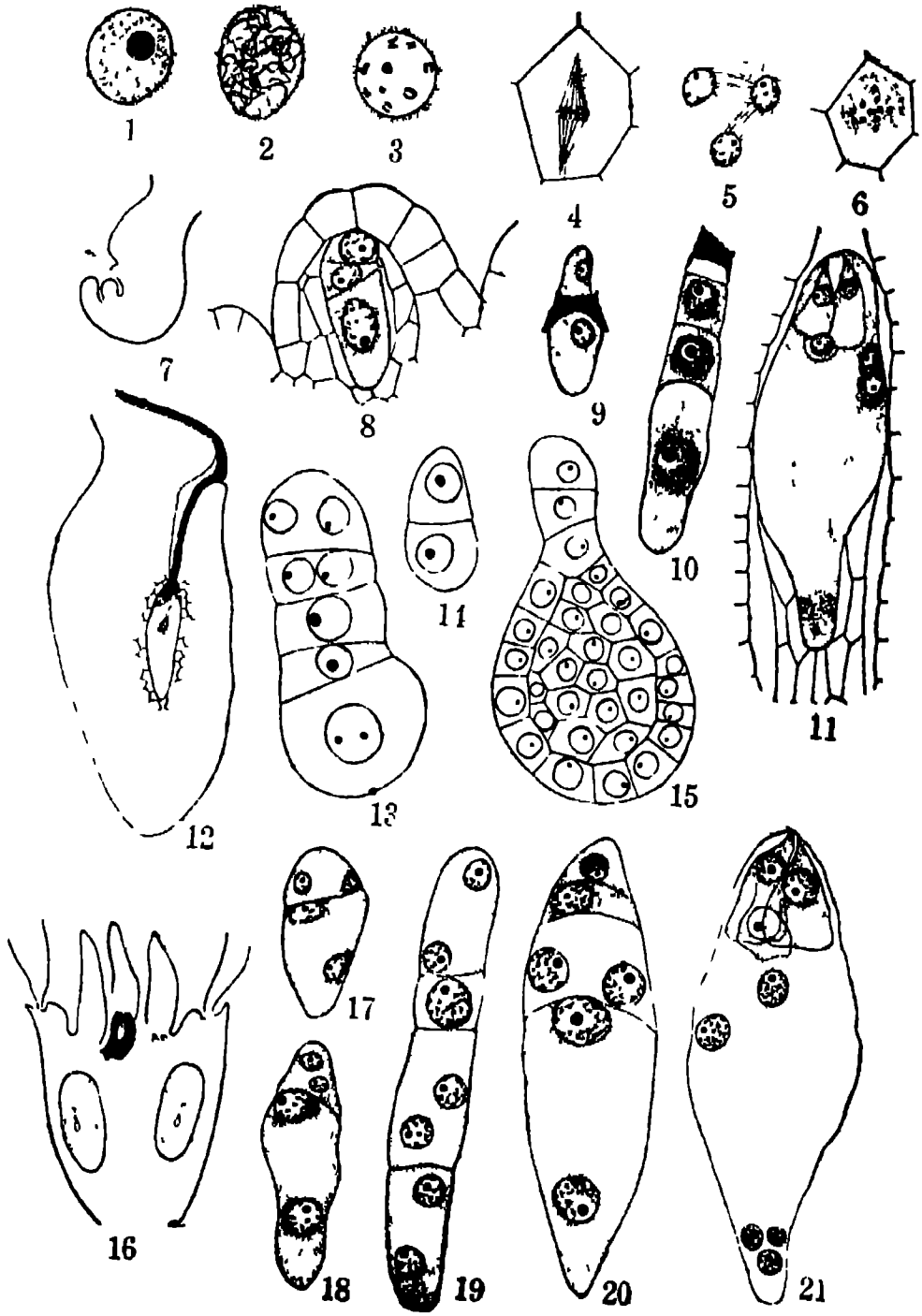
The nucleus of the pollen mother cell enlarges in size and becomes conspicuous by its staining reaction. The various phases of meiotic and mitotic divisions have been carefully followed (Figs 1–6, 22–24, 44 and 45). In the polar view of the metaphase plate, the chromosome numbers were determined as 11*n* and 12*n* for *P fruticosa* (Fig 6) and *H australiana* (Fig. 24) respectively. These were later on confirmed by the study of chromosome numbers in the root-tip Following nuclear divisions cleavage furrows with septa formation separate the four microspores These are enclosed for some time in the wall of the pollen mother cell and finally become dispersed. The microspores when mature distinctly show two coats, an exine and an intine which is thin In *H australiana* and *P fruticosa* two germ pores were observed The pollen grains show three nuclei at the shedding stage

Megasporogenesis

The ovary is inferior with a short style It is 4–5 locular in *P fruticosa*, *H australiana*, *P. pinnata* and *H venulosum* It is 10 locular in *B actinophylla* The ovules are pendulous anatropous and are found one in each locule. In *H australiana* in spite of the fact that four to five ovules are present only one or two develop further

The nucellar primordium becomes differentiated from the placental mass As development proceeds the ovules become anatropous, the micropyle being parallel to the funiculus. There is a single massive integument characteristic of the members of the *Umbelliflorae* The integument develops in the initial stages as a ring of cells at the base of the nucellus (Fig 26) In later stages it becomes massive The micropyle is very narrow and almost appears as a streak.

The development of the hypostase tissue has been noticed in *P. fruticosa* and *P pinnata* A similar case has been recorded in *Nothopanax arborea* by Piggot (1914). In addition Piggot mentions the occurrence of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an aril in the mature seed, the developmental stages of which were, however, not noticed. In the present investigation, the development of what resembles an aril (according to the description of Piggot) has been noticed in *P. fruticosa, H. australiana* and others The tissue referred to is the persistent obturator which is developed above the micropyle It is probable that Piggot observed this tissue in *Nothopanax arborea* and mistook it for an aril. The primordium for the obturator is formed as a mass of cells adjacent to the funicle (Fig. 7) The cells are rich in protoplasmic contents. They enlarge and arch over the micropyle (Fig. 12). In *H. australiana* the obturator tissue is glandular along the margin (Fig. 35) The obturator tissue to which the function of guiding the pollen tube has been ascribed is known to occur in quite a large number of families, being recorded in *Euphorbiaceae* (Maheshwari) *Labiatae* (Bushnell, 1936, Narasimhamoorthy, 1940) and in *Thymeleaceae* (Kausik, 1940). After the formation of the endosperm, the obturator tissue is crushed and gradually degenerates. Chalazal conducting strands which connect the vascular strands in the funiculus with the chalaza is present in all the members studied (Fig. 54) Such conducting strands have been observed in a number of families allied to the *Araliaceae* and in a number of other families which show no phylogenetic relation with the *Araliaceae*

The archesporium is differentiated simultaneously with the growth of the integument. It is mostly hypodermal and rarely sub-hypodermal in origin. A parietal cell which often remains without further development is formed (Fig. 8). Double archesporial cells have also been observed in all the plants studied, except *P fruticosa* (Figs. 26, 55) The suppression of the parietal cell formation has been recorded in some cases (Ducamp, 1902) where the archesporium directly functions as the megaspore mother cell

The occurrence of multiple archesporium is reported in many plants belonging to *Rhizophoraceæ* (Kersten, 1891), *Nyssaceae* (Horne, 1914) and Onagraceæ. Among the allied families its presence has been reported in *Cornaceae* (Hakansson, 1923; Horne, 1914 and Jonsson, 1881).

In the course of the development, the megaspore mother cell enlarges in size. The nucleus passes through the normal meiotic changes (Figs. 27 and 47) and forms the dyad. The next homœtypic division in the dyad results in the formation of a linear row of tetrad of megaspores (Figs. 10, 51). In normal cases the chalazal megaspore develops further and forms the embryo-sac accompanied by the degeneration of the other three megaspores (Fig 51). Enlargement of the micropylar megaspore with the degeneration of the other three has also been observed. Simultaneous development of

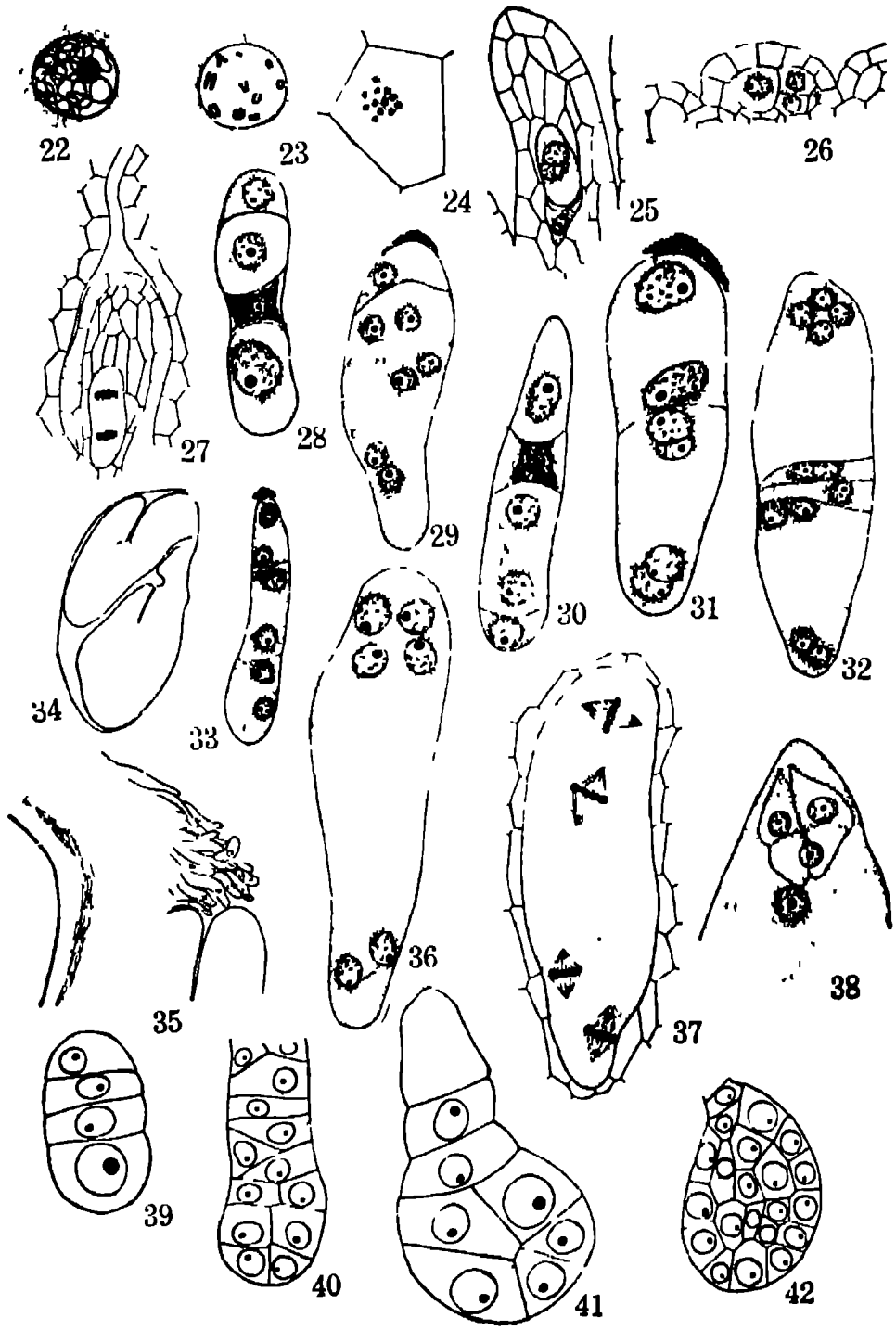
micropylar and chalazal megaspores as in *Oenothera* has also been noticed in one case (Fig 9).

The development of more than one megaspore upto the two-nucleate embryo-sac stage has been observed in *P pinnata* and *H australiana* (Figs 28–33). In rare cases they develop even upto the four-nucleate stage But in no case the development of more than one embryo-sac upto the eight-nucleate stage has been observed. Similar instances of development of all megaspores to certain extent have been seen in *Iphegenia indica* (Joshi, 1939), *Casuarina stricta* (Frye, 1903), *Gloriosa* and *Uvularia* (Afzelius, 1918) and in a number of Liliaceous plants Schniewind-Thies (1901) records such a feature for *Galtonia candicans*, *Convallaria majalis* and Stiffer (1925) for *Velthemia viridifolia*

The nucleus of the enlarging megaspore undergoes divisions and forms the complete eight-nucleate embryo-sac in the normal way (Figs. 20, 36, 37). The chalazal end of the embryo-sac in all the five plants studied tapers into a narrow pouch-like structure (Figs 11, 21, 50 and 60) In *H australiana* the nuclear divisions at the chalaza were belated, remaining only at the two-nucleate stage while at the micropylar end it was already four nucleate (Fig. 36).

The migration of all the four nuclei towards the micropylar end in the course of free nuclear divisions was noticed in many instances in *B. actinophylla* (Figs. 57–59). In such cases no nuclei were present at the chalazal end. It is not out of place to compare this stage with *Oenothera* type of development where only four nuclei are present in the mature embryo-sac But the comparison cannot be too far extended since the definite organisation of egg apparatus at this stage was not noticed.

Bisporic type of development as variations from the monosporic eight-nucleate type has been observed in a few cases in *H. australiana* and *P. pinnata*. Here, after the formation of the dyad, the homœtypic division was suppressed and the lower dyad cell enlarged and formed a two-nucleate embryo-sac (Fig 18) directly while the upper cell degenerated. In another instance both the dyad cells developed into the two-nucleate condition but the lower alone further developed (Figs. 17 and 25). It is quite evident that in the above-mentioned cases the wall formation is suppressed in either one or both the dyad cells and thus the tendency towards the bisporic type of development seems to be present The suppression of the homœtypic division following the formation of dyad and the consequent formation of a bisporic type of development has been recorded in the *Orchidaceæ* by Pace (1914), Afzelius (1916), etc. In these forms normal type of development is the rule and occasionally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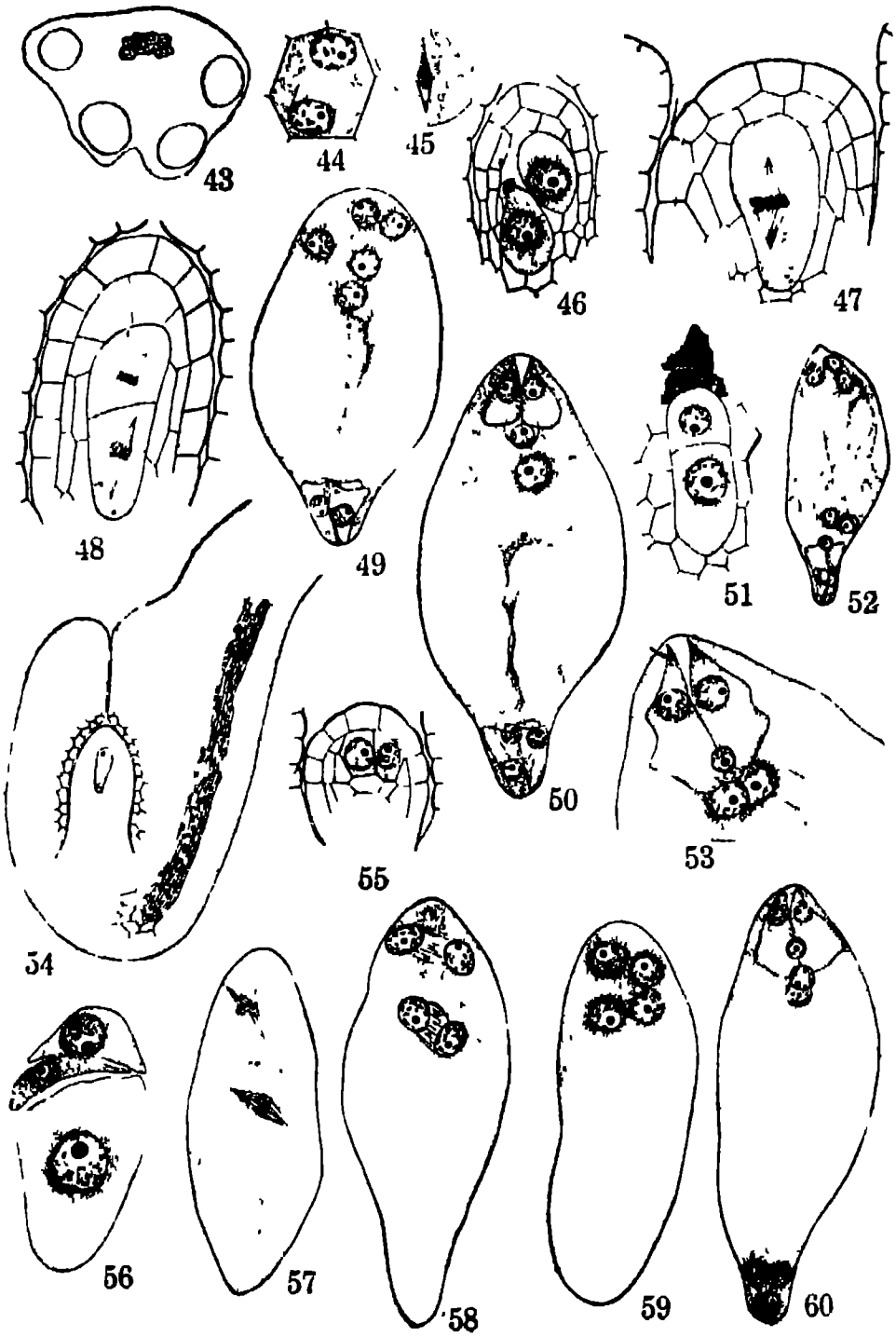
bisporic type of development arise as abnormalities Similar instances have also been reported in the *Oleaceæ* (*Syringa vulgaris*, Andersson, 1931), in the *Gesneriaceæ* (Cook, 1907) and in the *Liliaceæ* (*Iphigenia indica*, Joshi, 1939). Joshi cites further examples of this type of development from *Rutaceæ* (Mauritzon, 1935), *Cruciferæ* (Corti, 1930), *Nyctaginaceæ* (Rocen, 1927), *Myrtaceæ* (Greco, 1920), *Droseraceæ* (Smith, 1929) and the *Amaryllidaceæ* (Stener, 1925)

After the eight-nucleate embryo-sac is formed, there is the organisation of the nuclei into the egg apparatus, polars and the antipodals (Figs 11, 21, 50 and 60) In *H venulosum* the synergids are pear-shaped in the initial stages and develop beaklike structures later (Fig 53). In *H. australiana* the synergids are pointed at both ends (Fig 38) The polars have the tendency to be near the egg apparatus (Figs. 53 and 60) and very rarely migrate towards the centre They remain near together or closely adpressed for a long time (Figs 11 and 60) and fuse just before fertilization The antipodals remain nuclear in *P. fruticosa*, *H australiana* and *P pinnata*. In *H. venulosum* and *B. actinophylla* they form cells simulating the synergids in shape (Figs 49 and 50) A case of reversed polarity was noticed in *H. venulosum* (Fig 52) which was reported by the author (1943).

Fertilization is of the porogamous type and the entry of the pollen tube has been observed in *P. fruticosa* and *H venulosum* Before the pollen tube reaches the micropyle it glides along the surface of the obturator (Fig. 12). During its entry the pollen tube crushes one or both the synergids.

Stages in the embryogeny have been studied in *P fruticosa* and *H. australiana* A considerable time elapses between fertilization and the first division of the zygote The secondary nucleus undergoes free nuclear divisions after fertilization This is followed by the enlargement of the embryo-sac which gradually crushes the remaining nucellar cells and also the integument which remains as a thin papery layer The endosperm nuclei are large in size and at times contain more than one nucleolus. Nucleolar fragmentation is of common occurrence. In later stages wall formation takes place starting from the periphery and the cells are polyhedral Cells of the endosperm are studded with a lot of reserve food material possibly of the nature of starch

The first division of the fertilized egg is transverse. By further divisions a pro-embryo of four to five cells in a row is formed (Fig 39). The cell of the pro-embryo towards the chalaza develops further and forms the embryo. At the octant stage periclinal walls are formed separating off the dermatogen. In *P. fruticosa* the cells of the suspensor become embedded in the nucellar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tissue of the micropy e. The cells around it are devoid of contents This wedging of the suspensor cell into the nucellar tissue might procure nutrition to the developing embryo

Summary

1 A detailed account of the gametogenesis of some members of the *Araliaceæ*, viz., *Panax fruticosa, Hedera australiana, Polyscias pinnata, Heptapleuram venulosum* and *Brassaia actinophylla* is given in this paper. Embryogeny of *P fruticosa* and *H australiana* has been studied.

2 In the anther the hypodermal archesporium gives rise to the sporogenous layer and the primary parietal layer

3 Chromosome numbers for *P fruticosa* and *H. australiana* have been determined. The haploid numbers are 11 and 12 respectively.

4. The wall layer in *P. fruticosa* and *H australiana* is single. In *B. actinophylla* it is two-layered

5. The tapetum is uninucleate in *H australiana* but two-nucleate in *P fruticosa* and *B actinophylla*. Rarely it is three-nucleate in *B. actinophylla*

6. The ovules are anatropous and have a single integument.

7. In *H. australiana, P fruticosa and P. pinnata* there is the formation of an obturator.

8. A conducting strand is made out in the ovule.

9 The archesporium is invariably single but occurrence of double archesporial cells has been recorded in all plants studied except *P. fruticosa.*

10. The hypodermal archesporium cuts off a parietal and a megaspore mother cell.

11 The order of degeneration of megaspores is not regular but shows variations.

12. The chalazal megaspore usually enlarges and forms the embryo-sac. But the non-functional megaspores in *H australiana* and *P. pinnata* also develop upto the two-nucleate condition.

13 The development of the embryo-sac follows the normal-type but for certain variations.

14. In *P. pinnata* and *H. australiana* occasionally the development is of the bisporic type.

15. In *B. actinophylla* there is a tendency for the Oenothera type of development.

16. The antipodals persist and form cells in *H. venulosum* and *B actinophylla.*

17. The synergids are normally pear shaped, though in *H venulosum* they form beaks at a later stage

18. Fertilization is of the porogamous type

19 The endosperm is first nuclear and later wall formation sets in.

20 A rare case of reversed polarity in the embryo-sac was observed in *H. venulosum.*

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A PRELIMINARY NOTE ON THE EMBRYOLOGY OF *CASUARINA EQUISETIFOLIA*, FORST

By B. G L. Swamy

(*Bangalore*)

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(Communicated by Prof L S S Kumar, F A Sc)

The remarkable discovery of Chalazogamy in *Casuarina* by Treub in 1891 evoked very keen interest and initiated further studies of Casuarinaceæ and Amentiferæ from both morphological and anatomical points of view. Certain aspects of the megasporogenesis of *Casuarina stricta* was subsequently studied by Frye in 1903 and Juel (1903) recorded his observations on the origin and development of the female archesporium in *Casuarina quadrivalvis*. In spite of these contributions our present knowledge regarding the developmental stages in the life-history are far from being satisfactory. An investigation of several species of the genus has been taken up by the author and a few salient features in the life-history of *Casuarina equisetifolia* Forst have been embodied in this preliminary note

The archesporium of the microsporangium is subepidermal in origin and can be differentiated by rich cell contents and conspicuous nuclei After the formation of the endothecium, wall layers and tapetum, the microspore mother cells undergo the usual stages of the reduction divisions and form quartets of microspores arranged tetrahedrally. The quartets round off and their nuclei undergo division into tube and generative cells The pollen grains at the shedding stage are binucleate

Each ovary contains two erect ovules which arise laterally from a basal placenta (Fig. 1). The ovules are bitegumentary the inner integument differentiating slightly earlier than the outer; these grow upwards and organise a micropyle.

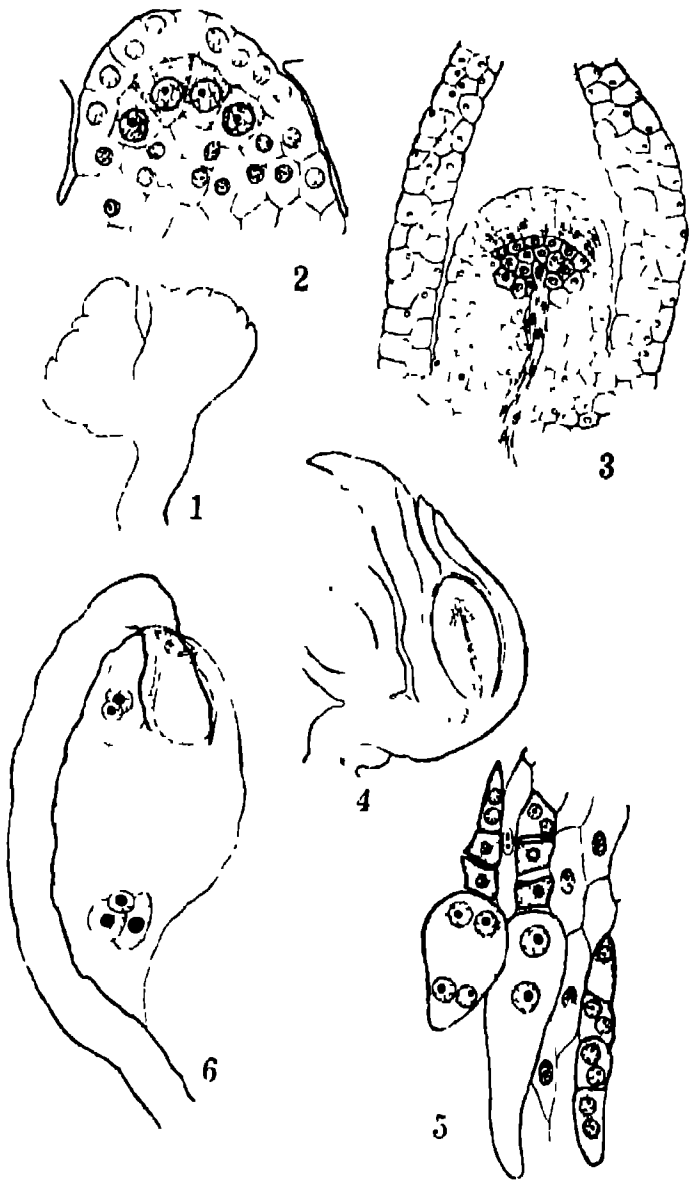
Simultaneously with the origin of the integuments a group of cells differentiate and become conspicuous in the subepidermal layer (Fig. 2); these divide transversely and the resulting cells towards the micropyle give rise to 5 or 6 parietal layers which make the sporogenous tissue deep seated; hand in hand with this the primary sporogenous layer also divides and increases in number. A conducting strand is organised (Figs. 3 and 4) at the chalaza and this extends upto the sporogenous mass. Some of the megaspore mother cells in the centre of the sporogenous mass also

contribute to the formation of the conducting strand instead of developing into embryo-sacs

The megaspore mother cells become elongated and undergo the usual meiotic divisions to give rise to a linear row of four megaspores (Fig 5) Simultaneous development of more than one megaspore of the tetrad is a very common phenomenon. The stages in development of the embryo-sac are quite normal and typical Usually at the two-nucleate stage, the embryo-sac elongates antipodally, grows into the chalaza in the form of a narrow long tube and terminally develops a small pouch The antipodal nuclei become surrounded by individual cytoplasmic sheaths and are very ephemeral, their position within the embryo-sac varies. The egg apparatus which is always organised at the micropylar end of the embryo-sac exhibits a variety of forms. The polar nuclei remain juxtaposed, occupying a position beneath the egg apparatus or somewhere in the antipodal elongation of the embryo-sac

The pollen grain germinates on the filiform stigma and the pollen tube grows down the style and after travelling through the tissues of the ovary, enters the chalaza and there branches profusely Ultimately the branch that contains the male nuclei makes its way between the antipodal tubular prolongations of the embryo-sacs on to the top of the particular embryo-sac that is destined to be fertilized (Fig 7) This course of the pollen tube differs from those reported by the previous workers on *Casuarina* investigated by them In those species, the general contention was that the pollen tube enters one of the antipodal swellings and travels within the embryo-sac to the bottom of the egg apparatus of that sac or that the pollen tube implants itself on any part of the embryo-sac membrane and discharges its contents into the sac at that point Such a feature is not noticed by the present author in the species investigated by him On the other hand, it was seen that the pollen tube follows a definite course in the nucellus and on reaching the micropylar tip of an embryo-sac, pierces the sac between the egg apparatus and the membrane of the sac and discharges its contents. One of the male nuclei fuses with that of the egg and the other one with the two unfused polars Thus a clear "double fertilization" is accomplished (Fig 6)

In spite of the fact that as many as 20 embryo-sacs develop to maturity within each nucellus, only one of them is fertilized, resulting in the formation of one embryo in each fruit It was observed that the triple fusion of the polars with the second male nucleus is in advance over that of the zygote. In some instances while the egg nucleus and the associated male nucleus



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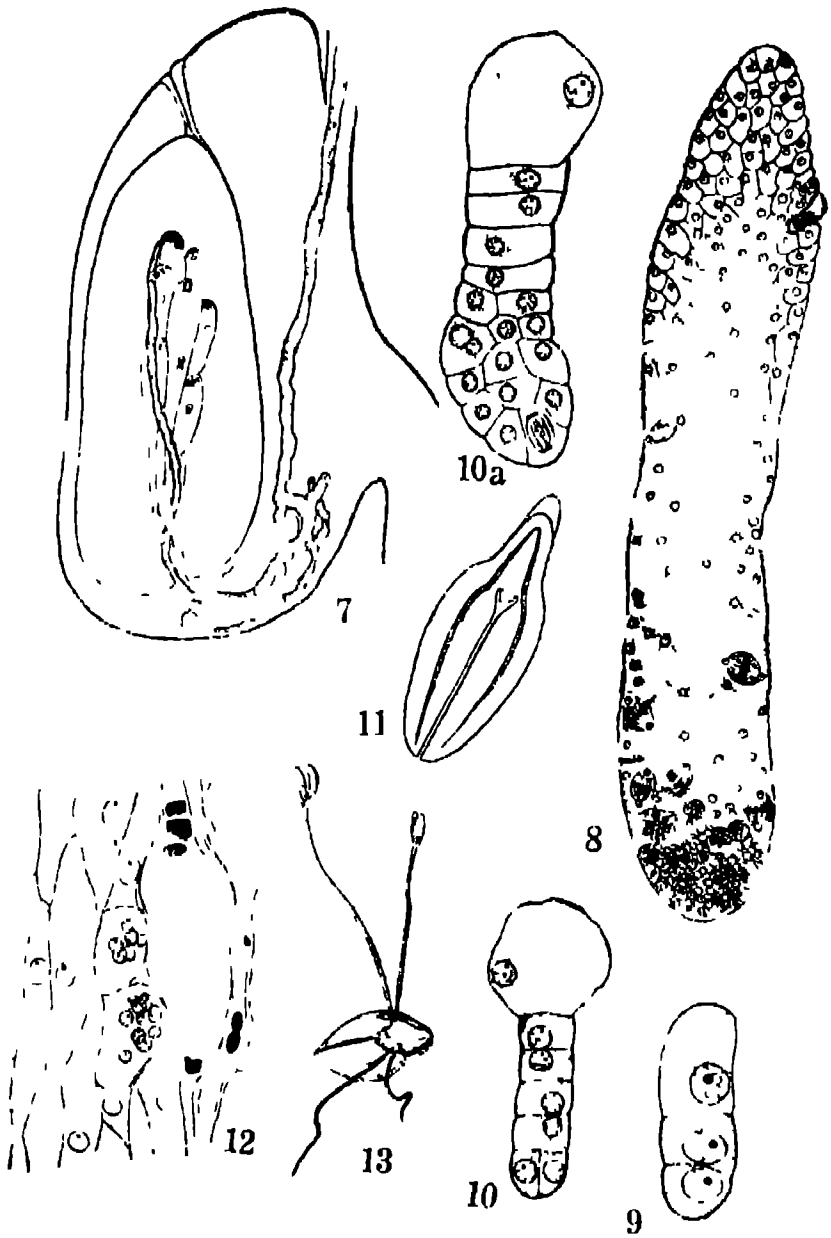

Figs 7-13—Fig 7 Double fertilization ×1800 Fig 8 A stage in the formation of endosperm with two-celled embryo. ×240 Fig. 9 Proembryo ×1800 Fig. 10 and 10a Stages in the development of the embryo Fig 10 ×1800 Fig 10a ×1260. Fig. 11 Longitudinal section of the mature embryo. ×40 Fig. 12 A stage in the formation of nucellar embryos. ×1260. Fig. 13 Germination of a polyembryonate seed. ×5.

were still at the stage of fusion, the primary endosperm nucleus had already divided four or five times

The wall formation of the endosperm commences from the micropylar end and proceeds centrapetally towards the chalazal end When the cellular endosperm fills the micropylar half of the embryo-sac, the zygote undergoes its first division. It is not unusual to find cellular endosperm above and free nuclear divisions taking place at the chalazal region of the same sac (Fig 8) Still later the endosperm tissue increases in bulk by intercalary divisions especially at the micropylar region around the embryo

The first wall laid down in the zygote is transverse A proembryo consisting of a linear row of three cells is organised (Fig 9) The basal cell enlarges and its nucleus gets hypertrophied The middle cell divides to form a suspensor consisting of four to five cells (Fig 10) The terminal cell undergoes the fisrt division by a vertical wall followed by quadrant and octant stages The mature embryo has well-differentiated radical, stem-tip and two cotyledons (Fig 11)

Casuarina equisetifolia often exhibits a range of variations which have not been recorded previously Two ovules sectioned from a particular tree invariably showed double nucelli with common integuments From the very begining the development of the female gametophytes in the two nucelli was similar and independent The two nucelli were in the majority of cases unequal in size After fertilization of an enbryo-sac in a particular nucellus was over, the second nucellus degenerated

Sometimes two pollen tubes enter the same ovule and double fertilization is accomplished in two embryo-sacs, resulting in the formation of two embryos in a single ovule. Occurrence of two proembryos side by side was noticed in some cases. Failing fertilization, some of the nucellar cells in certain ovules showed developmental stages in the formation of additional embryos (Fig. 12) Fig. 13 represents a case of the germination of a polyembryonate seed.

Further details and their relation to the systematic position of the family will be discussed in the full paper, to be published elsewhere

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INDIRECT STIMULATION OF UNSTRIATED MUSCLE

A Preliminary Communication

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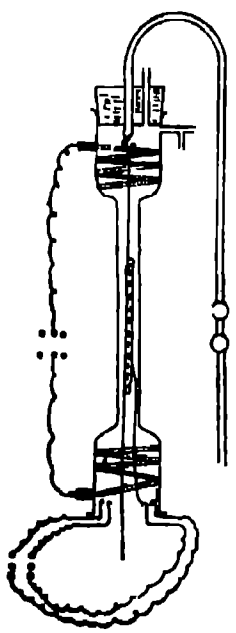
PLAIN muscle appears to be excited by two agencies difference in concentration of ions within and without the fibres and surface action When ions are added from without, the increase in concentration takes place outside the cells, when it is stimulated electrically or by increase in ismotic pressure, increase in concentration takes place inside the cells In this paper a preliminary study of the mechanism of surface action and that of nervous stimulation is presented For literature see review by McSwiney (1931)

Methods

Strips of dog stomach were isolated. The attached vagus nerve was isolated from the lower end of the œsophagus, the peri-arterial sympathetic may also be attached The muscle was placed in a chamber, so that it could be stimulated (*a*) chemically, (*b*) electrically with alternating or direct current, (*c*) or through the nerves In these experiments we have stimulated only the vagus by induction shocks for 15 sec. (Fig. 1)



Results

The muscle nerve preparation thus made was immersed in mammalian saline described previously (Singh, 1940) The responses obtained by stimulation of the vagus every 10 min. were quite regular and the muscle was responsive for several hours (10 A M to 8 P M.), the tension obtained being as high as 30–40 g. Only result obtained was contraction, which showed staircase and fatigue effects.

Relation to tone.—The response obtained by stimulation of the vagus was a twitch, the rate of rise and fall of which was somewhat less than that of the twitch produced by alternating current: relaxation may be prolonged like the direct current contraction in *Mytilus* muscle The vagus twitch was antagonistic to

tone If the muscle exhibited much tone it was inexcitable to vagus Just after the vagus twitch, tone was partially neutralised, showing, that, as with other forms of stimulation, the excitation by the vagus contained an inhibitory component. This does not necessarily mean that the vagus contains inhibitory fibres, because it is given by all forms of stimulation, such as by alternating current, potassium and acetylcholine

The above inhibition of tone is enhanced by increase of temperature, even though the respone may diminish; so that it is probably due to the liberation of calcium

Tone in the vagus-stomach preparation is thus myogenic The inhibitory component in the vagus excitation is necessary, for the efficient performance of the contraction, as inhibition of tone during the vagus contraction will be attended with diminution of viscosity. The dual action of the vagus is thus explained and the nerve is motor

A stimulus appears to consist of two components, one excitatory and the other inhibitory The excitatory component may be antagonised by (*a*) antagonistic excitation, such as increase of tone; (*b*) diminution of excitability; (*c*) adaptation; (*d*) drugs, or it may be made ineffective by the use of stimulus of sub-laminal strength Under such conditions inhibition will be produced, thus a relaxed muscle will contract, and a contracted muscle relax. This inhibitory component appears to be an integral part of the excitation process, so that a muscle may be excited to contract or relax. If this active process requires more oxygen than that producing contraction, then oxygen consumption will be increased, when the muscle relaxes, otherwise it will decrease

Effect of temperature.- In 3 experiments the optimum temperature was found to be 30° C ; as a matter of fact, at 37° C , the muscle may become inexcitable to vagus At 37° C., though the response becomes smaller, it is, however, more brisk than at 30° C. The optimum temperature for acetylcholine is also 30° C. In previous experiments (Singh, 1940), and by Winton, the optimum temperature for alternating current was found to be 24° C., but in the present experiments, it was 30° C , so mammalian tissues are also likely to vary in their properties.

Effect of calcium —Calcium is necessary for vagus stimulation, as with striated muscle nerve preparation of the frog. In 3 experiments, the optimum was found to be 0 05–0·10 M $CaCl_2$, more than that required for alternating current and same as that required for acetylcholine (0 05 M $CaCl_2$). With optimum concentration of calcium the responses may be very powerful.

Effect of potassium.—The optimum concentration of potassium is the same as that for alternating current (about 0·06 M KCl; 3 experiments). The muscle becomes inexcitable in excess of potassium.

Effect of hydrogen ions.—The optimum pH was found to be about 7·4–7·8 (3 experiments), somewhat greater hydrogen-ion concentration being necessary than that for alternating current For acetylchloine the same pH was necessary

Discussion

The stimulation produced by the vagus as well as acetylcholine does not belong to the potassium group; their properties resemble more those produced by alternating current However, it has not been possible to say whether it forms a group distinct from that produced by alternating current.

Summary

(1) The excitation produced by vagus stimulation has an inhibitory component.

(2) Tonus is antagonistic to vagus stimulation.

(3) The optimum temperature for vagus stimulation is 30° C

(4) The optimum concentration of calcium is about 0·05–0·07 M CaCl, potassium about 0·06 M KCl and hydrogen ions, pH 7·8–7 4

(5) The stimulation produced by vagus and acetylcholine does not belong to the potassium group. It resembles more that produced by alternating current

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EXCITATION IN UNSTRIATED MUSCLE

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Excitation phenomena in unstriated muscle are very complicated Various kinds of contractions and inhibitions occur (Singh, 1936; 1937; 1938 *a, b, c, d, e, f*; 1939 *a, b*; 1940, 1942 *a, b*, 1943 *a, b, c, d, e, f, g, h, i*, 1944 *a, b, c*; Rao and Singh, 1940, Singh and Mrs Singh, 1943, 1944; Gokhale and Singh) It would be very helpful if the inter-relation between these contractions is made clear by the help of a comparatively simple hypothesis or model

In unstriated muscle there are certain salient features concerning excitation These are. (1) The mechanisms of excitation produced by electric current and by ions appear to be different Increase in permeability decreases the sensitivity to the former and increases that to the latter. (2) The excitation by ions depends upon the production of a difference in their concentration on two sides of the muscle membrane (Straub, 1903, 1907) or increase in permeability, or their attachment to sensitive patches (Clark, 1935) Certain substances, however, act in too small a concentration to produce such a difference (4) Certain ions may produce inhibition at one time and excitation at the other (5) Calcium may produce effects similar to those of increase of osmotic pressure (6) Certain agencies affect the two excitabilities similarly and the others oppositely (7) Change of length produces contraction. (8) The muscle shows withdrawal contractions There are several other phenomena, which need elucidation.

It is the object of this paper to correlate these phenomena

Excitation in unstriated muscle appears to be produced by difference in concentration of ions within and without the fibres.

In considering difference in ionic concentration on two sides of the muscle membrane causing excitation, the next question is whether it is the difference or the ratio. After the tension has subsided with a certain concentration of potassium, addition of a greater concentration produces further tension, and if the concentration of potassium in successive doses increases in arithmetical progression, the tension in successive stimulation is less than the preceding. This would be evident from consideration of the ratio R_o/R_i, where R_o is the concentration of ions outside, R_i that of

the ions inside. A numerical increase in the numerator and the denominator by the same amount would result in decrease of the ratio

Difficulty arises in explanation of certain phenomena If the tension produced by addition of potassium to the outside of the muscle fibres depends upon the ratio R_o/R_i, then the less permeable the membrane of the fibres, not only should it become more excitable to alternating current but also to potassium, and the tension would be maintained longer But generally in *Mytilus* muscle when the excitability to alternating current was high, it was inexcitable to potassium (0 1 M KCl). Addition of potassium did not produce any tension, as if it was not added at all, apparently something prevented potassium from coming into a zone where a difference in concentration of ions would be produced without and within the fibres This would be explained if the muscle was surrounded by a second membrane with properties more or less similar to the first, dividing the muscle into two zones, inner and outer Increase in tension would result only if there was a difference in concentration of ions in the outer and inner zones respectively. The outer membrane would require to be more permeable than the inner Two zones have been described by Roskin 1925, 1926; Carleton (1943)

The next question is whether the outer zone is aqueous or non-aqueous The postulation of a sodium space and a potassium space has been described by Singh (1939 *a*) The outer membrane would then be permeable to sodium and potassium, but the inner membrane impermeable to sodium. The outer zone must however be non-aqueous; this assumption is based on the previous assumption that the sodium contraction would be caused by the entrance of sodium or chloride ions in the outer zone, thus disturbing the normal ionic equilibrium If the concentration of sodium ions is already equal to that in the medium than no such increase can take place Hence, this outer zone must be non-aqueous, otherwise the membranes would have to support a large difference of osmotic pressure

This is in agreement with the views of Beutner, Osterhout (1936) who have postulated a non-aqueous zone for the muscle

The above idea is also supported by the fact that the stimulating power of the monovalent cations varies in the same order ($Li < Na < NH_4 < K$) as their velocity in protoplasm as found by Osterhout. The stimulating power of potassium is much greater than that of ammonium, and though its velocity in aqueous solutions is practically equal to that of ammonium, in protoplasm it is 40 times greater This suggests that the outer zone is non-aqueous

As the outer membrane is more permeable than the inner anything that increases the permeability of the muscle, would at first make the latter

more excitable to potassium and less to alternating current With great increase in permeability the muscle would become inexcitable to both Thus in the absence of calcium *Mytilus* muscle becomes less excitable to alternating current and more to potassium With time like frog muscle, it becomes inexcitable to both

The presence of the outer zone is also suggested by the action of barium and sodium (Singh, 1944 *b*).

The presence of the outer zone also explains the increase in excitability following increase in permeability to ions The more permeable the muscle to an ion, more quickly its concentration will be raised in the outer zone.

If the muscle be immersed in a potassium rich solution, then R_i will be increased, to rise to the stimulating concentration, more time will be required for R_o. Thus potassium will diminish the response and increase the latent period, as happens with barium and sodium contractions, as well as the stretch and release contraction, which is presumably caused by sodium chloride (Singh, 1938 *b*)

The relative impermeability of the inner membrane would make it comparatively impermeable to ions with lesser penetrating power, such as sodium chloride, anions, barium, compared to faster moving ions such as potassium and ammonium. The former ions will therefore produce continuous contractions, as is found experimentally.

Ions will not only affect R_o, R_i, but also the permeability of the membranes Thus calcium has effects resembling that of other ions, that is producing contraction, potentiating the response to potassium and decreasing that to alternating current At the same time it has other effects that affect the two excitabilities similarly. If calcium decreases the permeability, potassium has probably an opposite effect, accounting for the lasting inexcitability in excess of potassium. This explains the two factors affecting excitability (Singh, 1939*b*).

Interaction between Excitation and Inhibition

For the sake of simplicity, the excitatory and the inhibitory action of ions may be termed excitation and inhibition respectivily As the potassium contracture is antagonised by increase of osmotic pressure and alternating current, excitation inside is antagonistic to excitation outside and *vice versa* Similarly the two inhibitions are antagonistic (Singh, 1942 *b*). As inhibition is the opposite of excitation, it follows then that increase of inhibition outside will increase excitation inside This is realised by experiment. In frog stomach ammonium has an inhibitory action. In the presence of

ammonium the contraction produced by increase of osmotic pressure is greater (Singh, 1939 *b*).

It thus follows that inhibition inside will increase excitation outside Diminution of excitation inside will also have the same effect. In *Mytilus* muscle, this can be done in three ways. (1) by diminishing the concentration of potassium by immersion in hypotonic solution; (2) by replacement of potassium by sodium; and (3) by immersion in lithium saline (Singh, 1944 *b*). All these procedures increase the excitability to potassium and diminish that to alternating current. The presence of sodium or chloride inside the muscle fibres is the basis of all tonic contractions.

Tonic Contractions

Tonus —This would be due to the presence of sodium in the outer zone as well as the inner Plain muscle would then contain more sodium or chloride than striated muscle Dog stomach exhibits greater tone than frog stomach and is comparatively richer in sodium (Gokhale and Singh)

Sodium will have a second action, that on muscle viscosity (Singh, 1943 *h*) This is in agreement with the fact that plain muscle is more viscous than striated muscle Continued stimulation increases the sodium content of skeletal muscle (Fenn, 1936) as well as viscosity (Levin and Wyman, 1927)

Tonic contractions produced by alternating current.—Increased sensitivity to ions outside during stimulation, or their entrance in the outer zone will have the following effects· (1) Diminish primary tension as with A.C and D.C. contractions; (2) account for the optimum voltage for A.C , as with increased voltage ions outside begin to antagonise the alternating current; (3) diminish adaptation, as the anions are antagonistic to the action of calcium, producing adaptation to adaptation or accommodation to accommodation (Singh, 1943 *d*); (4) may actually produce a tonic contraction or inhibition with passage of current for long duration (Singh, 1942 *b*) (5) diminish the rate of relaxation either by causing after-excitation or increasing the viscosity. Some of these effects will not be produced if the action of ions outside is inhibitory as in frog stomach

Ordinarily the action of ions outside antagonises the action of ions inside, and this results in (1) diminution of the primary tension; (2) decrease of the rate of relaxation If the action of ions outside is very powerful, then the primary contraction produced by alternating current may be entirely neutralised and a tonic contraction produced by the current from the outset, instead of after sometime. If this happens then the primary tension will increase with decrease in the rate of relaxation. This is actually found

to be the case. Thus barium and cyanide are the two most powerful stimulants, and in their case, not only the rate of relaxation decreases, but the primary tension produced by the current also may increase (Figs 1, 2).

A.C. off-contracture -In *Mytilus* muscle the primary tension may completely subside during the passage of alternating current On cessation of the current a contraction is produced, having the properties of the potassium contraction; the relaxation may be rapid. This shows that it is produced by ions outside the fibres This phenomenon is produced by ions that increase the permeability of the muscle. The explanation appears to be that during stimulation, ions leak into the outer zone and cause subsequent stimulation on cessation of the current (Fig 3)

But it also happens that there is no decline of tension during the passage of the current and the off-contracture is still produced, but its relaxation is very slow This then cannot be solely due to leakage of ions from the inner zone, but can be explained as due to leakage of ions into the outer zone both from the inner zone as well as the exterior, as this is more marked if stimulating anions are added to the saline (Figs 2, 4)

Slow relaxation of plain muscle--This is due to the entrance of ions in the outer zone during stimulation (Fig 4) This view is supported by the fact that it is produced by all substances that increase the permeability, and

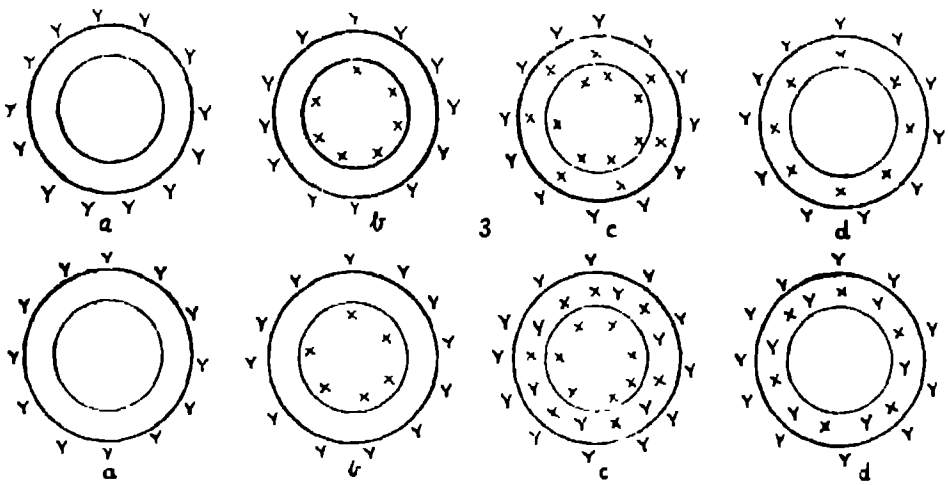

FIG. 3 Schematic representation of the A.C off-contracture (*a*) Resting State (*b*) Increased concentration of ions inside the fibres produced by A C stimulation (*c*) Leakage of ions to the exterior (*d*) The A C off-contracture

FIG. 4 Schematic representation of the A C off-contracture (*a*) Resting muscle (*b*) A C. stimulation (*c*) Leakage of ions (*d*) The A C off-contracture.

inhibited by calcium which decreases the permeability. The entrance of ions in the outer zone produces two things. (1) They may cause stimulation after cessation of the stimulus as occurs with veratrine, (2) or just increase the viscosity and decrease the rate of relaxation. Continued frequent stimulation causes contracture and increases the viscosity, as well as the sodium content, as is found both in striated and in unstriated muscles. Veratrine decreases the electrical resistance of nerve (Guttman and Cole 1942).

Contraction produced by stretch and release (Singh, 1938 *b*, 1943 *d*) —As this contraction has properties of the sodium chloride contraction, it is produced by sodium or chloride. It appears that sudden change of length alters the permeability and sodium chloride enters the outer zone and causes excitation. The muscle appears to have greatest permeability at a certain length, and sudden approach to this length from either side causes contraction, the muscle has an optimum length for the potassium contraction.

This leads to an important law which may be enunciated as follows— The action of any factor which increases the sensitivity of the muscle to excitation by ions without the fibres, when suddenly intensified, evokes a contraction, the properties of which are the same as those of a contraction produced by excitation by ions without, the converse of this is not true.

Mechanical and other effects.—Anything that injures the outer membrane will make it more permeable and thus cause contraction hence the production of mechanical and injury contractions or inhibitions. Chemicals may injure the membrane; hence substances like ether may cause contraction. Lack of oxygen will produce similar effects, as energy will be required to maintain comparative impermeability. Hence a temporary increase in excitability to potassium might be expected (Singh, 1938 *b*) or a temporary increase in tonus (Magnus, 1904, Mickuliez-Rodecki and Luez, 1924).

Correlation of the Theories of Ionic Concentration and Surface Action

Difference causing Contraction and that of Clark —Drugs are active in such minute concentrations that their action could not be due to a difference in concentration within and without the fibres. But just as stretch or release sensitises the muscle to ions outside, so it is possible that some of the drugs produced their effects not *per se*, but through the agency of other ions by sensitising the muscle to these ions, the mechanism of this sensitisation being an increase of permeability (Singh, 1943 *g*).

Correlation of the Colloid Theory (see Heilbrunn 1937) *and the permeability theory* —These two theories are not mutually exclusive. Thus ions probably

enter the muscle during stimulation or liberated in active form under certain conditions, and may either cause contraction or increase the viscosity, the latter must be due to action of these ions on the colloids

Adaptation

If excitation is associated with the entrance of ions into the outer zone, then inhibition must be associated with an opposite change The action of calcium on *Mytilus* muscle is suggestive. In its absence the muscle gains weight and base, in its absence the swelling is reversed It is probably the latter action which makes the ions leave the outer zone.

Adaptation is probably produced by liberation of calcium ions in the outer zone, excluding other ions from it, thus diminishing R_o The decrease in R_o will diminish tone, and increase the excitability to A C , but if the concentration of calcium increases further, then R_i will increase, causing diminution in excitability to A.C as well

Two agencies diminish tonic contraction in plain muscle (*a*) calcium; (*b*) increase in osmotic pressure The former decreases R_o and the latter increases R_i, the result being diminution of the ratio R_o/R in both instances

Inhibition

The fact that potassium causes inhibition or excitation in the same muscle, shows that the factor determining inhibition or excitation resides, not in the stimulus, but in the cell To produce inhibition, however, ions have to enter the outer zone so that for inhibition an increase of permeability would be necessary, and this would be followed by a decrease, produced by inhibition *per se*

Spontaneous or Rhythmic Contractions

These occur when the excitability of the muscle is intermediate between that which induces continuous tension, and that which induces no tension, that is, between great excitability and inexcitability If excitability to excitation from without is dependent upon permeability or affinity of muscle colloids for ions, then for spontaneous contractions an intermediate permeability or affinity is necessary, as shown by the following observations:—

(1) Heart muscle contains sodium intermediate in amount between that contained in striated muscle and that in unstriated muscle Isolated striated muscle is relaxed, cardiac muscle is in spontaneous contractions, and unstriated muscle is in tone.

(2) *Mytilus* muscle is more permeable to potassium than to sodium, with the result that if sodium chloride produces spontaneous contractions, potassium produces continuous tension.

(3) The muscle is more permeable to thiocyanate than to bromide, and more to the latter than to chloride thiocyanate gives continuous tension, bromide may give spontaneous contractions, and chloride may not excite the muscle

(4) The muscle is more permeable to potassium than to ammonium, and more to latter than to sodium There might be no tension with sodium, spontaneous contractions with ammonium, and continuous tension with potassium

(5) There might be no tension in *Mytilus* saline containing 0 02 *M* $CaCl_2$, spontaneous contractions with 0 01 *M* $CaCl_2$ and continuous tension in the absence of calcium, calcium decreases permeability to sodium

(6) If no contractions are produced in the presence of 0·01 *M* $CaCl_2$, spontaneous contractions may be produced in the absence of calcium

(7) Excess of potassium might produce spontaneous contractions in 0·02 *M* $CaCl_2$ and continuous tension with 0 01 *M* $CaCl_2$; calcium decreases premeability to potassium

(8) The muscle is less permeable to ions with decreasing pH. Caffeine might produce no tension at pH 6, spontaneous contractions at pH 7, and continuous tension at pH 7 8

(9) Barium like potassium might give spontaneous contractions in 0·01 *M* $CaCl_2$, and continuous tension in the absence of calcium decreases permeability to barium

Spontaneous contractions in *Mytilus* muscle can be explained on the basis of liberation of calcium by ions (Singh, 1938 *f*, 1944 *a*) Ions enter the outer zone, and thus cause excitation Their presence liberates calcium so that the ions then leave the outer zone This in turn causes calcium to recombine, so that ions again enter the outer zone, and so the cycle is repeated.

The reason for an intermediate permeability for these contractions is then understood. If the permeability is less, the muscle will be inexcitable; if the permeability is great, then the adaptation factor will be neutralised or overcome so that continuous tension will result

The above phenomena are compared to partial defence of a place If the defence is strong, then the attacking forces will not be able to penetrate defence positions. If the defence is rather weak then the attacking forces will penetrate, but will be repelled by counter-attack each time If the defence is very weak, then the attacking troops will overcome the defence entirely.

Summary

Excitatory phenomena in unstriated muscle can be explained if it is assumed that the muscle consists of two zones, outer and inner, and excitation be due to difference in concentration of ions in these two zones. Moderate increase in permeability would diminish the excitability to alternating current and increase that to potassium; great increase would diminish the excitability to both. An increase in the permeability of the outer membrane by physiological action, injury, asphyxia would cause excitation or inhibition. Spontaneous contractions are caused by increase in permeability, not great enough to cause continuous tension. Substances to which the muscle is moderately permeable, such as sodium and barium, produce continuous tension as they are unable to enter the inner zone. Substances to which the muscle is more permeable such as ammonium or potassium produce only a temporary contraction.

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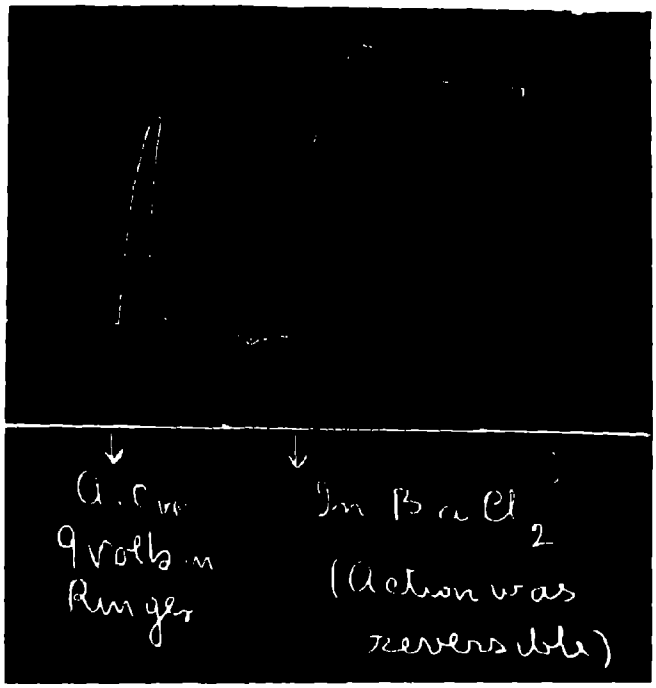

FIG. 1 *Mytilus* muscle Stimulation with A.C. 9 V in 0.02 M BaCl

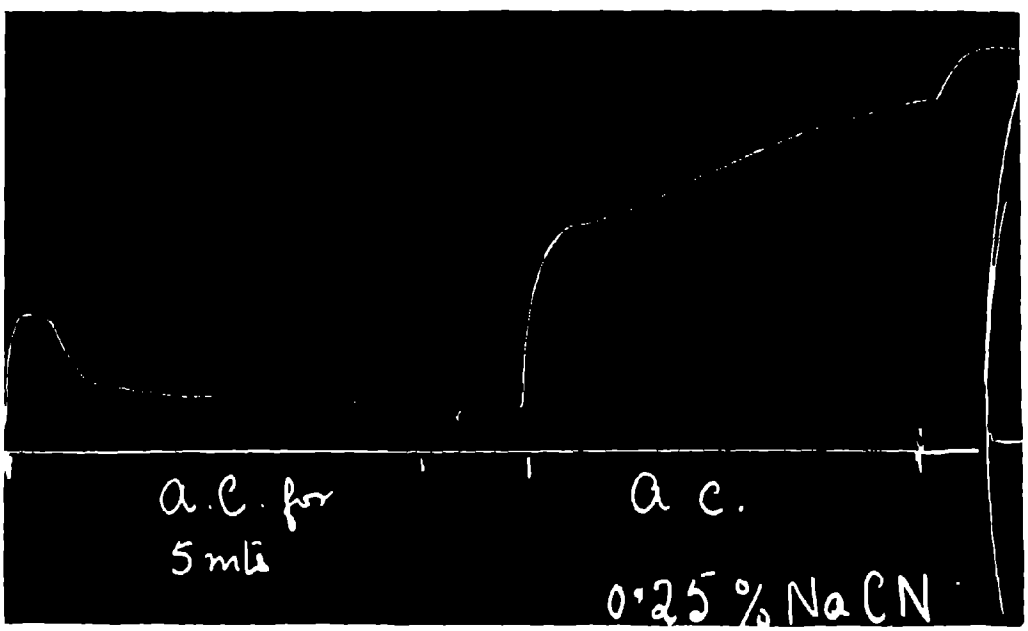

FIG. 2 *Mytilus* muscle Stimulation with A.C. in NaCN

ON THE RELATIONSHIP OF RHODUSITE TO GLAUCOPHANE

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Rhodusite is a soda-amphibole which was first collected in 1889 by Bukowski (1889, 226) from Rhodus Island, Asia Minor Specimens of this were sent by him to Baron Foullon for investigation Foullon had the mineral analysed and he named it rhodusite (1894, 176) He considered the mineral to be a variety of glaucophane, and this early description has been followed more or less by later writers Iskull (1908, 387) refers to it as an alumina-free ferric glaucophane According to Murgoci (1915, 633), the mineral belongs to the extreme ferric end of the glaucophane series. Hintze (1897, 1260) has listed this mineral under glaucophane So has Dana (1911, 46), and this has been repeated in Dana's Text-book revised by Ford (1932, 577), where rhodusite is stated to be a fibrous variety of glaucophane

A consideration of the chemical and optical properties of rhodusite and glaucophane, however, reveal several points of difference In Table I, the four existing analyses of rhodusite are given, together with four analyses of glaucophane published by Kunitz (1930, 244)

Table I

	1	2	3	4	A	B	C	D
SiO_2	55·06	54·01	54·38	55·06	57 73	56 97	56 77	55 47
Al_2O_3	0 49	0·23	0·28	0·18	12 04	10 83	11 28	12·18
Fe_2O_3	15·48	15·70	15 12	14·54	1·16	2 92	1·89	1·62
FeO	7·40	9·42	9 21	7 17	5·41	8 27	10 84	13 73
MgO	11·49	10 01	10·54	12·30	13·02	10·43	8·92	7·36
CaO	0·98	1·52	1·23	1·17	1 04	0 68	1 24	0·38
Na_2O	6·38	6·22	6·86	6·52	6 98	6 79	6·45	6·70
K_2O	0·80	0·35	0·31	0·23	0 68	0·65	0·60	0·82
H_2O	1·98	2·25	2·16	2·44	2·27	2·23	1 93	2 01
MnO		0·14	0·11	0 09				
	100·06	99·85	100·20	99·70	100·33	99·77	99 92	100 47

1 Rhodusite.—H B Foullon "Uber Gesteine und Mineraie von der Insel Rhodus," *Sitz Math-natur k.k Akad. Wiss*, 1894, 100, I Heft, Abt. I, 174

2, 3, 4. Rhodusite —W Iskull, "Uber den Rhodusit vom Flusse Asskys (Bergbezirk Minussinsk in Sibirien) Beiträge zur Kenntnis seiner chemischen Constitution und Verwitterung," *Zeits Kryst*, 1908, 44, 371–74

A, B, C, D Glaucophane —Quoted from W. Kunitz, "Die Isomorphieverhältnisse in der Hornblendegruppe," *Neues Jahrb* 1930, B. B. 60, Abt. A, 244

It will be seen from this table that rhodusite contains a very small percentage (less than 0 5 per cent) of Al_2O_3, whereas in glaucophane it is very high (in analyses A and D it is over 12 per cent) The other marked variation is in the Fe_2O_3 content; in rhodusite it is about 15 per cent whereas in glaucophane it is very much less, rarely reaching 3 per cent; while in many glaucophanes this is less than 1 per cent (Kunitz, 1930, 198) It does not seem quite satisfactory to get over these differences by merely designating rhodusite as an alumina-free ferric glaucophane

These differences are also brought out clearly by calculating the number of metal atoms of each kind on a basis of 24 (O,OH,F). In Table II, these calculations have been set out, and it will be seen from this that while there is close correspondence in the values for all the other atoms, Al and Fe''' show great differences

TABLE II

	1	2	3	P
Si	7·94	7 88	7·93	7 83
Al	0 09	0 03	0·05	1 89
Fe'''	1 68	1·72	1 65	0·20
Mg	2 48	2·19	2·30	2 06
Fe''	0 89	1·15	1 12	1·10
Mn		0·02	0 01	
Na	1·78	1 75	1 94	1 81
Ca	0 16	0 24	0 19	0·12
K	0·15	0 05	0 05	0·12
(OH)	1 92	2 19	2 10	1 94

1, 2, 3 Rhodusite —Correspond to the first three analyses given in Table I

P Glaucophane —Average of the four glaucophane analyses given in Table I

Al falls between the Si group and the Mg group and according to Warren (1930, 198) may be expected to replace either Si or Mg. From Table II it is seen that in rhodusite Al and Si together form 8 atoms, whereas in glaucophane Al is in excess and replaces partly Si and partly Mg.

In their study of the composition of the alkali amphiboles, Berman and Larsen (1931, 142) have given the ratio of Mg : Al : Si in a triangular diagram on the assumption that the sum of these three is constant, any variation from the total of 13 being considered by them as due to experimental error. By noting the positions where the greatest concentration of analyses are found, these authors have determined the most common amphibole types. This diagram is reproduced in Fig. 1, and in it are plotted the position of the eight analyses given in Table I. It will be seen that while the glaucophane analyses correspond very nearly to the position given to

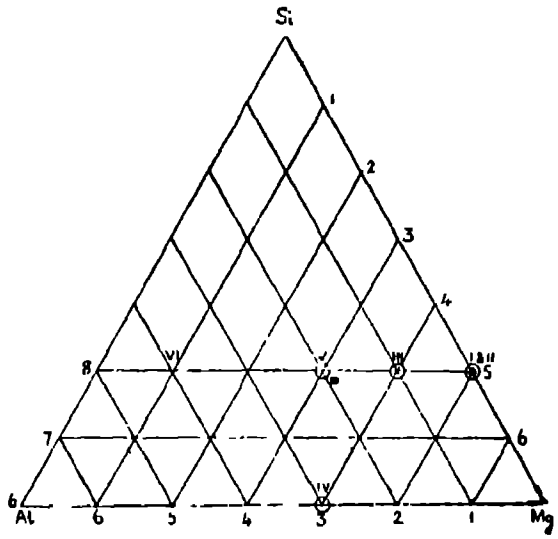

FIG 1 Composition of the Amphiboles (after Berman and Larsen) Mg-Al-Si Ratios

I Tremolite, II Soda-Tremolite, III Arfvedsonite, IV Hastingsite, V Glaucophane types, VI Glaucophane (?) The small inked square represents the position of the glaucophane analyses, and the small inked triangle, the position of the rhodusite analyses given in Table I

the glaucophane types by Berman and Larsen, the rhodusite analyses occupy a different position

When the optical properties of rhodusite and glaucophane are compared, the differences between these minerals become still more apparent

In the case of rhodusite there appears to be some contradiction between the descriptions of Iskull and Murgoci Iskull (1908, 373–74) states that in the first two analysed specimens of rhodusite, the optic sign is negative, and the extinction *not* $Z \wedge c$, whereas according to him, the optic sign of the third specimen may be positive or negative According to Murgoci (1915, 632), the sign of the mineral appears to be positive, and his statement that the sign of elongation of rhodusite is positive is followed by a question mark, for he thinks that he might have made a mistake in this determination. In a later paper, Murgoci (1922, 426) states that the sign of the mineral is negative. Niggli (1926, 473) gives the optic plane and Z as perpendicular to 010 and the angle of extinction as $X \wedge c$ The optic orientation of this mineral is also diagrammatically represented in Fig. 216 of Niggli's Mineralogy (1926, 471).

Glaucophane has its optic plane parallel to 010, the extinction is $Z \wedge c$, the sign of the mineral is negative, and the sign of elongation positive. It

is clear from this that the optic orientation of glaucophane is quite different from that of rhodusite.

Rhodusite cannot, therefore, be considered as a variety of glaucophane, because both in chemical composition as well as in optical characters there are fundamental differences between the two minerals

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THE SWELLING OF UNSTRIATED MUSCLE PRODUCED BY CERTAIN IONS AND ITS RELATION TO PERMEABILITY, EXCITABILITY, ABSORPTION AND SECRETION

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Plain muscle undergoes changes in weight when immersed in certain solutions, the order in which these changes take place, bears a close resemblance to that in which these solutions produce physiological reactions (Singh, 1938 *a*, *b*, 1942, Rao and Singh, 1940) In the present paper some of these changes are further described and their significance discussed

The muscle used has been the plain muscle from *Mytilus edulis*

Results

Effect of potassium When the muscle is immersed in isotonic solution of potassium chloride, it swells and gains base, that is, potassium (Table I), the potassium enters as chloride. The swelling and gain of base is diminished by calcium (Table II)

Table I

Effect of soaking the muscle in sodium and potassium salines

Sodium of *Mytilus* saline replaced with potassium Assuming that the water content of unsoaked muscles is 78 p c the concentration of base in the muscle is calculated if the ionic concentration was the same within and without the muscle The calculated values are compared with those actually found, a discrepancy shows that the muscle is alive and the permeability intact Base m eq /gm of wet muscle Pairs of muscle from same animal compared soaked for 16 hours

Number of Muscle	In sodium saline				In potassium saline				Percentage increase in base
	Base per gram	Percentage increase in weight	Concentration	Isotonic concentration	Base per gram	Percentage increase in weight	Concentration	Isotonic concentration	
1	0 317	5	0 319	0·450	0 788	70	0·463	0 490	148
2	0·314	5	0·300	0·430	0 811	88	0 431	0·496	158
3	0·261	−0·5	0·260	0·440	0·740	91	0·398	0·496	145
4	0·305	9	0·280	0 460	0 913	97	0 463	0 500	199
5	0·310	−5	0·320	0·430	0·722	77	0 408	0·492	132
6	0·373	9	0·350	0·460	0·798	87	0 426	0 495	117

TABLE II

In potassium saline; effect of calcium (0 02M $CaCl_2$)

Recalculated from Singh and Mrs Singh (1944)

Number of Muscle	Ca				-Ca				Percentage increase in base
	Base per gram	Percentage increase in weight	Concentration	Isotonic concentration	Base per gram	Percentage increase in weight	Concentration	Isotonic concentration	
1	0·774	77	0 437	0 490	0 963	109	0 46	0 507	24
2	0 545	78	0 306	0 49	1 175	156	0·45	0 513	115
3	0 749	78	0·420	0 49	1·120	152	0·44	0 50	47
4	0 660	55	0·430	0·47	0·957	96	0 48	0·49	45
5	0 604	41	0 43	0·45	0 921	125	0 41	0 50	52
6	0 858	96	0 44	0 490	1 131	137	0·47	0 50	31

The concentration of base in most muscles remains less than that of the medium, especially in the presence of calcium, showing that the muscle is alive and the permeability intact In some muscles the concentration of base becomes almost equal to that of the medium, showing that the normal permeability of the muscle is destroyed. this concentration of potassium greatly decreases the impedance (Singh and Mr Singh, 1944 *a b*) Calcium appears to protect the membrane against the action of potassium

Effect of ammonium – The effect of this is greater than that of sodium, but less than that of potassium (Tables III, IV) In these tables the concen-

TABLE III

Effect of Ca-*free sodium and ammonium saline on base content*

No of Muscle	In sodium saline		In ammonium saline		Percentage increase in base
	Base per gram	Percentage increase in weight	Base per gram	Percentage increase in weight	
1	0·290	10	0·524	43·1	80
2	0·359	2	0·438	42·2	22
3	0·311	0 5	0·389	1	25
4	0·314	5	0·499	32	59
5	0·272	– 2	0·444	27	3
6	0·301	5	0·500	35	66

TABLE IV

Effect of Ca-*free ammonium and potassium saline on base content*

No of Muscle	In ammonium saline		In potassium saline		Percentage increase in base
	Base/gm	Percentage increase in weight	Base/gm	Percentage increase in base	
1	0 504	17	0·788	87	56
2	0 502	32	0·650	34	29
3	0·502	35	1·224	100	144
4	0 510	38	0·736	90	44
5	0·439	30	0·711	81	85
6	0 454	42	0 740	85	63

tration of base can be calculated from the increase in weight, it is much less than that of the saline.

Effect of sodium.—The effect on the base content of various sodium salts has already been given in a previous paper (Singh and Mrs Singh, 1944). The concentration remains below that of the saline, in iodide, thio-cyanate, however, it may approach that of the saline Effect of cyanide is shown in Table V, the concentration of base in the muscle is the same as outside

TABLE V

Effect of Ca-*free sodium cyanide saline on weight and base content*

Number of Muscle	In sodium chloride				In sodium cyanide				Percentage increase in base
	Base per gram	Percentage increase in weight	Concentration	Isotonic concentration	Base per gram	Percentage increase in weight	Concentration	Isotonic concentration	
1	0·432	15	0·38	0·45	1 526	178	0·55	0·51	253
2	0·484	26	0·38	0 47	1·192	189	0·41	0 52	146
3	0·487	20	0 40	0·46	1·268	151	0·50	0·51	160
4	0·485	25	0·38	0·47	1 473	176	0·53	0·51	204
5	0 386	14	0·33	0 44	1·515	177	0·54	0 51	289
6	0 401	15	0·35	0 45	1·368	161	0 52	0 52	241

The effect of calcium on that of sodium chloride is reversible.

Effect of lithium.—In calcium-free lithium saline, though the muscle increases in weight, the base content becomes less than in sodium saline (Table VI). The presence of calcium does not appear to make much difference to the base contents in lithium saline (Table VII).

TABLE VI

Effect of Ca-free lithium saline on base

No of Muscle	In lithium saline			In sodium saline			Percentage increase in base
	Base per gram	Percentage increase in weight	Concentration	Base per gram	Percentage increase in weight	Concentration	
1	0·186	14	0 17	0 392	27	0·31	110
2	0·212	10	0·20	0 466	53	0·31	119
3	0·186	15	0·16	0·603	58	0 38	278
4	0 373	63	0 23	0 701	84	0 38	88
5	0·375	6	0·35	0 479	22	0·39	28
6	0 443	11	0·40	0 513	40	0 37	151
7	0 187	13	0 16	0 477	20	0 39	155
8	0 250	18	0 21	0·266	9	0 25	8
9	0 293	33	0 27	6 370	12	0·33	26
10			0 20			0 14	
11			0 43			0 17	
12			0 39			0 15	

TABLE VII

Effect of calcium (0 02 M $CaCl_2$) *on the base content in lithium saline*

No of muscle	Concentration of base	
	Ca	−Ca
1	0·221	0 166
2	0 182	0·218
3	0·188	0 215

Effect of divalent ions In increase in base occurs in the order Ca < Sr < Ba, but the increase in weight occurs in the order Ba < Ca < Sr (Tables VIII, IX)

TABLE VIII

Effect of calcium and strontium salines

Number of Muscle	In calcium saline				In strontium saline				Percentage gain in base
	Base per gram	Percentage increase in weight	Concentration	Isotonic concentration	Base per gram	Percentage increase in weight	Concentration	Isotonic concentration	
1	0·205	−10	0·205	0·59	0 328	15	0·28	0·60	60
2	0·178	− 9	0·20	0·58	0·285	15	0·24	0·60	60
3	0·270	− 5	0·28	0·60	0·367	24	0·28	0·60	36
4	0·066	− 4	0·07	0·60	0·287	20	0·23	0·60	300
5	0·099	1	0·10	0·56	0·302	20	0·25	0·60	300
6	0·080	−12	0·11	0·56	0·280	24	0·22	0·60	300

TABLE IX

Effect of strontium and barium salines

Number of Muscle	In strontium saline				In barium saline				Percentage increase in base
	Base per gram	Percentage increase in weight	Concentration	Isotonic concentration	Base per gram	Percentage increase in weight	Concentration	Isotonic concentration	
1	0 260	16	0·22	0·60	0·376	− 9	0·41	0 56	44
2	0·305	13	0·27	0·60	0·551	8	0·52	0·60	80
3	0·236	14	0·20	0·60	0 418	− 3	0 43	0·55	77
4	0·217	13	0 20	0·60	0 482	− 7	0·52	0 56	122
5	0·255	26	0 20	0·50	0 346	3	0·36	0·55	35
6	0 253	18	0 21	0·60	0 348	−15	0 43	0·52	37

Effect of blotting —Blotting increases the gain in weight (Table X).

The increase in weight during shorter intervals of time follows the same order, as in 16 hours. In divalent cations Ca, Ba, the muscle at first loses weight

TABLE X

Effect of blotting on increase in weight/percentage increase
Muscle soaked in pure isotonic solution of barium chloride

Time of soaking hours	Pairs of muscles					
	1		2		3	
	Blotted	Unblotted	Blotted	Unblotted	Blotted	Unblotted
0	0	0	0	0	0	0
1	−17·5		−13		−18	
2	−13·9		10·4		−15·6	
3	− 8·8		1·7		− 2·3	
4	− 3·3		5·7		− 0·3	
6	2·1		10·6		8·1	
7	0·6		24·6		16 9	
18	17·5		46·5		40·2	
19	18·2		46·5		41 8	
20	20·5	1·5	48·8	2 5	45 9	1·2

Effect of hydrogen ions —The swelling is greater in alkaline than in acid solution (Table XI). In pH 4·6-4 4 however, though the muscle loses weight, the base content increases. In some muscles, the concentration of base may become same within and without the muscle; this is due to the death of the muscle, as shown by the muscle becoming opaque.

TABLE XI

Effect of hydrogen ions on gain of base

Number of Muscle	pH 5·6 (phosphate)				pH 7·8 (phosphate)				Percentage increase in base
	Base per gram	Percentage increase in weight	Concentration	Isotonic concentration	Base per gram	Percentage increase in weight	Concentration	Isotonic concentration	
1	0·370	7	0 35	0 45	0·532	29	0 41	0·46	44
2	0·425	6	0 40	0 44	0 602	51	0·40	0 48	10
3	0 378	18	0 33	0 47	0·469	29	0 38	0 46	24
	pH 4 4 (acetate)				pH 7 8				
1	0·363	7	0 40	0·45	0 503	36	0·37	0·46	39
2	0·421	−8	0·46	0·45	0·559	38	0·40	0·46	33
3	0·371	−5	0 40	0 44	0·685	60	0 43	0 46	85

Discussion

The gain of ions by the muscle is due to the attraction of the muscle colloids this is shown by the fact that in the presence of calcium the swelling is reversed-syneresis. Such a phenomenon cannot be explained on the basis of permeability of membrances Further the swelling appears to be caused of living as well as dead muscle In sodium cyanide the concentration of base is equal to that of the medium, thus showing that the normal permeability is destroyed. Probably, the muscle is dead but this cannot be stated with certainty, as the muscle heated above 50 C. does not swell In all other solutions, the muscle appears to be living as shown by the deficiency of base This is also shown by the fact, that these changes begin in a few minutes, when the muscle can be restored to its normal activity, if it had become inexcitable in the solutions

This attraction of the muscle colloids for the ions, undoubtedly accounts for the accumulation of potassium in cells, and the entrance of ions against a concentration gradient. It must be due to froces of the Donnan equilibrium, as well as capillary electric forces The cell colloids probably contain small pockets of fluid, or capillary alleys, wherein presumably are located the laboratories of life

Such colloidal phenomena, probably form the basis of absorption and secretion. At one end of the cell, the colloids attract, and at the other end they repel. This alone is however not sufficient The cell must be bounded

by membranes allowing unidirection permeability as might occur in nerve, owing to its rectifying properties

The mechanism for one substance say, sodium chloride may be imagined as follows The cell attracts sodium chloride, the permeability of the membrane at one end of the cell being to allow the passage of the sodium chloride form without inwards, and at the other end from within outwards The increased concentration of sodium chloride within the cell, would liberate calcium, as has been assumed to occur in plain muscle This would cause syneresis, and so the sodium chloride would pass out of the other end of the cell, or the membranes may be made alternately impermeable by liberation of calcium

As the sodium chloride leaves the cell, the stimulus for the liberation of the calcium, would cease to exist, and so the calcium would recombine and absorption of sodium chloride would be resumed Absorption and secretion would thus be rythmic –the calcium pump

It is interseting to note that substances which form insoluble compounds with calcium, are not absorbed (Wallace and Cushny, 1898)

The gain of base and weight by the muscle are governed by two factors, the rate of absorption of ions by the muscle colloids, and the rate of the passage of ions through the membrane The observed rate of change measures the slower of the two processes

There are resaons to believe, that the change in the base content is a measure of the permeability of the muscle membrane These are

(1) When *Mytilus* muscle is immersed in a hypertonic solution of sodium chloride, it swells and gains base This swelling is prevented if the concentration of calcium is increased (Singh, 1938*a*) the muscle then behaves like an osmotic cell and loses weight in hypertonic saline [Calcium (0 01–0 02 M $CaCl_2$)], thus, decreases the permeability to sodium chloride

(2) The diminished base content in lithium saline is due to the outward passage of ions, this might be due to the lesser affinity of the muscle for lithium, or to impermeability of the muscle to lithium, the diffusable ions passing out. In both instances, however, the muscle should lose weight These changes can be explained if the muscle colloids have affinity for water, and the muscle membrane less permeable to lithium than sodium or potassium It appears that increase of permeability brought about by absence of calcium is still not enough for lithium to pass through

The action of barium is the opposite that of lithium the muscle gains base, though it loses weight. It can only be explained on the assumption that the muscle is permeable to barium

In solutions of pH 4·6–4·4, though the muscle loses weight, it gains base. In some muscles the concentration of base becomes the same within and without the muscle, owing to the death of the muscle. The increase in base therefore is clearly due to increase in permeability, the increase in hydrogen ions, as shown by the effect of pH 5·6, having the opposite action on colloids

(3) In isotonic solutions of monovalent cations in calcium-free solutions, which are likely to damage the muscle membrane, the rate of increase of weight is linear at first, and then increases before its final diminution (Fig 1) This

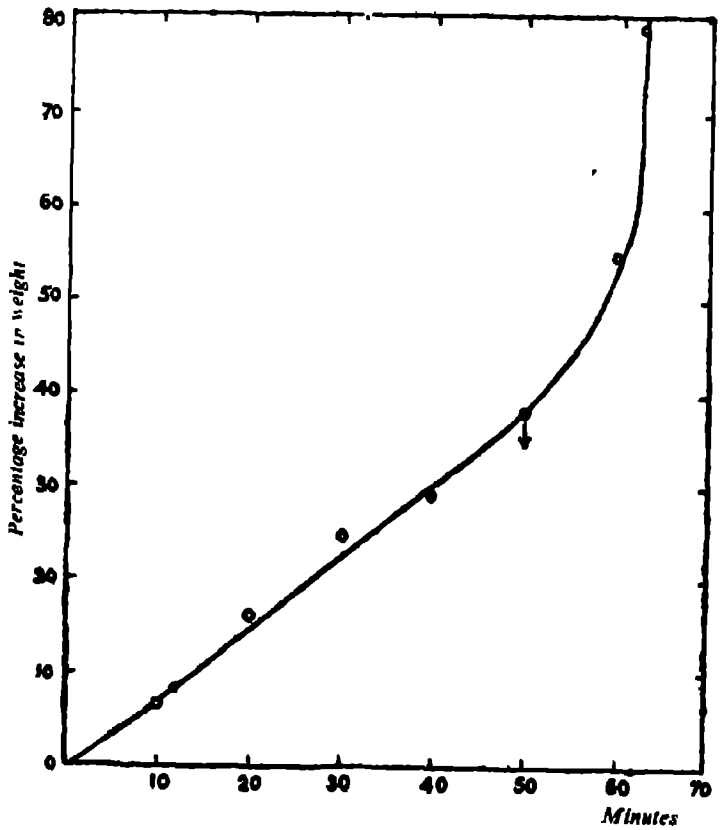

FIG. 1. The swelling of *Mytilus* produced by isotonic potassium chloride, in the absence of calcium

does not occur, or occurs less frequently in the presence of calcium. The explanation appears to be that the solution damages the muscle membrane thus increasing the permeability, and so hastening the entrance of ions In isotonic solution of potassium chloride, the muscle membrane appears to

give way in about 30–50 min, but in the presence of calcium it appears to remain intact for 9 hours

(4) The effect of blotting can only be a surface action, and appears to increase the permeability; the impedance of frog muscle is decreased.

In *Mytilus* muscle there is another contributory factor to the swelling Part of its osmotic pressure is maintained by a non-electrolyte, which is indiffusable so that if the muscle is placed in solution of ions to which it is permeable, then it will gain base and water, and swell in hypertonic solutions. The swelling would then be determined purely by permeability considerations

The rate of entrance of ions appears to be determined by the permeability of the membrane. From the effect of barium and pH 4 6–4 4, it appears that physiological activity is affected, not in the same order as the swelling, but the change in the base content produced by ions, that is the permeability of the membrane. The more permeable the membrane to an ion, the greater the exciting power of the latter

The mere presence of an exciting substance outside the fibres is not sufficient to cause excitation, as shown by the action of barium and sodium The latent period of the barium contraction may be as long as one hour; this time is sufficient for the substance to diffuse into the inter-fibre spaces. The passage of electric current for 2–3 sec or addition of adrenaline, or stretching the muscle is sufficient to initiate the barium contraction It would then appear that the current probably carries the ions into the muscle membrane, and adrenaline increases the permeability (Singh, 1943); change of length has a similar action

Ions which are more excitatory than others may also be more inhibitory, and inhibition and excitation may be affected identically, so that one kind of inhibition depends upon increase in permeability This correlation between excitation and inhibition can be explained as produced by entrance of ions, excitation being a direct effect, and inhibition an indirect one, due to liberation of calcium. The greater the number of ions entering the muscle, the greater will be the liberation of calcium, so that inhibition will be enhanced by increase in permeability, the muscle may then be excited to relax.

Substances that cause dehydration, cause inhibition; this might be due to increase in concentration of ions within the fibres, the action being akin to that produced by increase in osmotic pressure.

The action of hydrogen ions, pH 4 6-4·4, suggests that substances that damage the muscle may cause contraction by increasing the permeability,

if the muscle is in good condition Hence substances like ether may cause contraction, and so also asphyxia.

Summary and Conclusions

Mytilus muscle gains base when immersed in solutions of cations in the order $Li < Na < NH_4 < K$; $Ca < Sr < Ba$ The physiological activity varies not in the order of the swelling produced by ions, but the gain in base. This gain of base appears to be determined by permeability. As the exciting and inhibitory power of ions varies in the same order, it is therefore concluded that excitation or inhibition depends upon a common factor, such as the permeability of the membrane It is suggested that the mechanism of absorption and secretion by cells depends upon its colloidal reactions.

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THORAX (*TH*)—AN AUTOSOMAL GENE IN *DROSOPHILA PSEUDO-OBSCURA*

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Introduction

Amongst the progeny of pair matings of *Minute* (*M*) flies several males and females with unequal wings were observed by Miss Rowena Lamy of the Institute of Animal Genetics. Edinburgh, in February 1937. These when bred together gave rise to a stock with a high percentage of abnormal flies. Careful study of the abnormals showed that the irregularities were not confined to wings alone as observed by Miss Lamy, but a small percentage of flies also appeared showing defects of the thorax and legs, as well. It was decided therefore to name this new mutant "thorax" (*th*).

Description

The visible effects of *thorax* are on the general structure of the thorax and its appendages and may thus be classified under two heads:

1. Effects on the thorax 2. Effects on the appendages.

The latter may be further considered under two sub-heads:

(*a*) Effects on the wings and halters (*b*) Effects on the legs.

1. The effect on the thorax is less constant than it is on the wings; only a small percentage of the *thorax* flies show defective thorax. In less extreme cases of defective thorax, a median groove may be seen on the dorsal surface of the thorax dividing the thorax into two halves. The groove may be deep with the two halves quite separate. In extreme cases, one of the halves gets reduced in size or may even be totally absent; the head of the fly in such cases droops over the reduced or missing half. Deformities in structure can also be noticed amongst the micro and macro setæ on the thorax. The former may show stunted growth while the latter are frequently bent at an angle not unlike the condition of bristles obtained in *blunt* (*bl*) of *D. pseudo-obscura*

2 (*a*) Perhaps the most remarkable and in certain respects unique peculiarities are seen in the wings of *thorax* flies. The size, shape and structure of the wings may be subject to considerable modification and alteration or both. Ordinarily only one of the wings shows the deformity.

Instances where both the wings are deformed are not numerous. In the simplest cases, shape and structure remain normal and only size of the wing is modified The modified wing is held slightly raised at an angle and can be easily marked from the normal wings The wing may be distended with fluid only, presenting an inflated cushion-like appearance or with fluid and air bubbles giving a blistered appearance. The general shape and size of the wing too may at the same time undergo profound changes. In extreme cases, the whole wing is completely absent

Modification in wing structure may take the form of presence of extra veins or of duplication in parts or of the whole wing The duplication when slight is confined to a vein or two It may extend to the main roots of the vein and may cause duplication both in the radius and in the auxillary sclerites (Figs 1 and 2) In extreme cases of duplication there are two wings· (i) growing separately side by side, facing the same direction and joined only at the proximal portion (Fig. 1) or (ii) two wings not growing separately but facing each other, one half being the " mirror image " of the other half and having a common and continuous outer margin (Fig. 2). This type of wing duplications may be termed as "mirror image" duplications as distinct from ordinary duplications. I have come across in this stock of an extra-ordinary instance of " triple " wing. The wing belongs to the first order of extreme duplications with two distinct wings, but the anterior wing shows " mirror image " duplication, while the posterior is normal (Fig. 3).

Duplications may occur on both sides. Duplicated wings are larger than the normal and are held stretched out.

Halters.—One or both halters may be absent. In some instances they are enlarged resembling the character of similar name or held drooping down resembling the character *balancer down* of *D. pseudo-obscura.*

(*b*) *Legs.*—Very rarely flies are obtained with one of the third pair of legs missing Apart from this, no other visible abnormality associated with thorax has been observed

All the above-mentioned characteristics are inherited together. All attempts at getting pure stocks of one or more of the many abnormalities, free from the rest of the abnormalities, have so far failed It is possible therefore that all the observed peculiarities are due to one and the same gene.

Genetical

thorax ♀ ♀ when mated to *wild type* ♂♂ yielded all normal individuals. When an F_2 was raised, *thorax* again appeared in the offspring, proving that it is a simple autosomal recessive.

Failure to get a " pure " breeding stock.—Though selective inbreeding has been going on for nearly one and half years, a pure breeding *thorax* stock is not yet available. A certain percentage of normal flies invariably appear amongst the progeny. The percentage no doubt varies from culture to culture, but on an average about 30% of the flies obtained form the pair matings of *thorax* are normal. The record of flies obtained from 55 pair matings of *thorax* is given below

No of cultures	Total number of flies examined	Total number of *th* flies obtained	% of *th* flies
55	3425	2445	71·39

Are the normal flies really normal or are they the result of overlapping of thorax with wild type ?—To test this point, virgin *normal* and *thorax* females from stock were isolated and mated to brothers having same appearance and their offspring separately recorded The results are tabulated below:—

No of cultures	Total number of flies examined	Total number of *th* flies observed	% of *th* flies
1 *Cross* "Normal" virgin *thorax* ♀♀ × "Normal" *thorax* ♂♂			
19	1346	956	71·03
2 *Cross* "Abnormal" virgin *thorax* ♀♀ × "Abnormal" *thorax* ♂♂			
36	2079	1489	71·62

The percentage of *thorax* flies obtained in both the crosses is practically the same showing that the normal looking flies obtained in *thorax* stock are *genetically thorax*.

Location of thorax (th)

In a preliminary experiment carried out to find the chromosome of *thorax*, *thorax* ♂♂ were outcrossed to *vermilion, purple, tangled, arristopedia* (*v pr tg arr*) ♀♀ and an F_2 raised. Out of the 1789 F_2 offspring (817 ♂♂ ; 972 ♀♀) examined no recombination with *pr* was obtained It was assumed therefore that the gene for *thorax* is linked to the same group of mutants as *pr*, namely the III group.

Location of *thorax* was then carried out by first making up a stock—*orange plexus thorax* (*or px th*); *or px th* ♂♂ were then crossed to *wild type* virgin ♀♀ and the normal F_1 ♀♀ backcrossed to *or px th* ♂♂ from stock. The recombination data obtained is given below:—

CROSS.—

$$\frac{or\ px\ th}{+\ +\ +}\ ♀ \times \frac{or\ px\ th}{or\ px\ th}\ ♂ \text{ (26 pair matings)}$$

	Genotype	♂♂	♀♀
Non-cross overs	*or px th*	398	443
	Wild type	450	527
Single cross overs	*or*	102	99
	px th	79	99
	or px	25	27
	th	3	8
Double cross overs	*or th*	1	
	px	1	5

As only 71 39% of the *thorax* flies look phenotypically *thorax*, correction had to be made in considering the percentage of recombinations of *thorax* with *orange* or *plexus*. The corrected recombination percentages of *thorax* with *or* and *px* are 22·4 and 3 1 respectively. *px* gives with *or* 17·5% recombination which means *th* is to the right of *px* and the order of the genes is *or px th*.

Thorax is therefore located at 22 4 units to the right of *orange*, *i.e.*, if *or* is at zero. But recent work of Bhattacharya (1938) has placed *or* at 1 0 which would mean that *th* is at 23·4 on the III chromosome

Discussion

Many genes are now known in *Drosophila* that produce changes in more than one character Mullar's classical analysis of the *truncate* wing series in *Drosophila melanogaster* has shown that different mutant genes at the same locus may cause either a shortening of the wing, an eruption on the thorax, a lethal effect, or any combination of two or more characters. Dobzhansky (1929) noted that ten out of the twelve different mutants differing in characters as eye colour, wing size, etc., of *Drosophila melanogaster* examined for shape of spermathecæ, showed distinct differences. Similar manifold effects of a single gene were observed by Savelieu (1928) in *vestigial* flies of *D. melanogaster*; *vestigial* flies had reduced halters, modified location of post-scutellar bristles, decreased productivity, another variability in the number of egg tubes and delayed development, Prabhu (1939) in

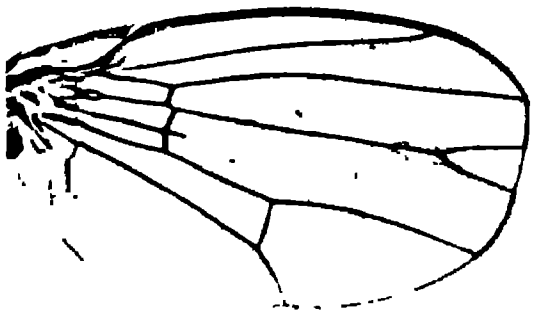

Fig. 1. Microphotograph of wing showing ordinary duplication

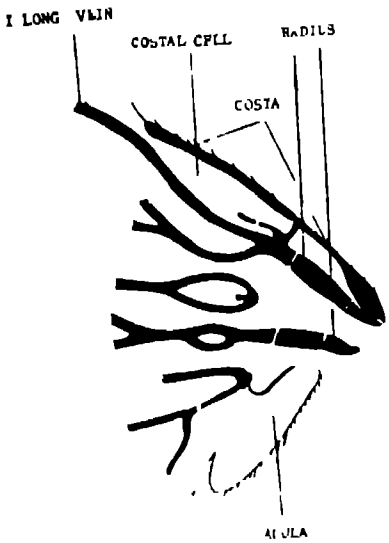

Fig. 1*a*. Wing base of ordinary duplication. Note the two distinct radii and their branches.

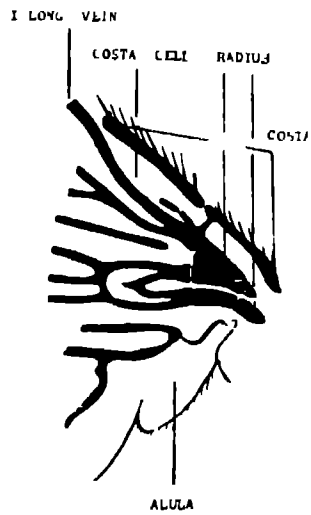

Fig. 1*b*. Wing base of ordinary duplication. Note the three radii and their branches. First two radii are fused.

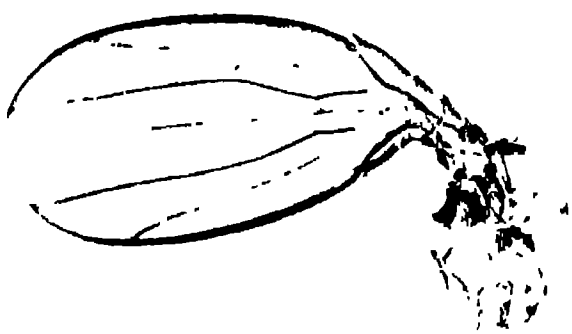

Fig. 2. Microphotograph of wing showing mirror image duplication

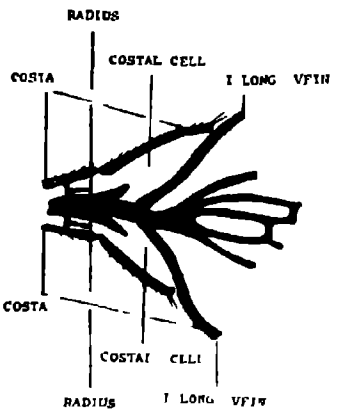

Fig. 2*a* Wing base of "mirror image" duplication. Note the double costa, costal cell and other veins. Radii though double are not distinctly separated

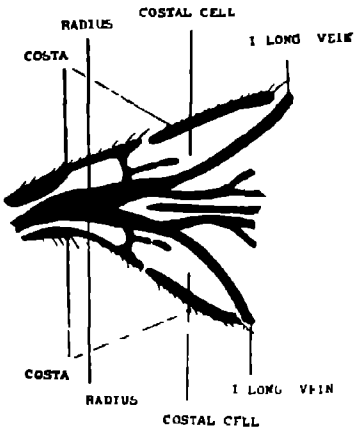

Fig. 2*b* Wing base of "mirror image" duplication. Differs from Fig. 2*a* in having clearly distinct radii

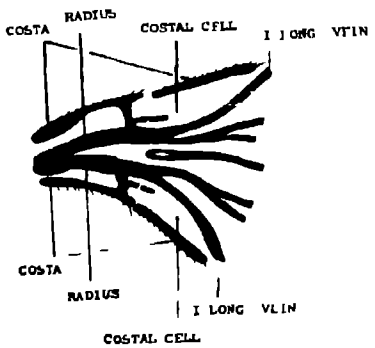

Fig. 2*c* Wing base of "mirror image" duplication. Radii more clearly marked out than in Fig. 2*b*

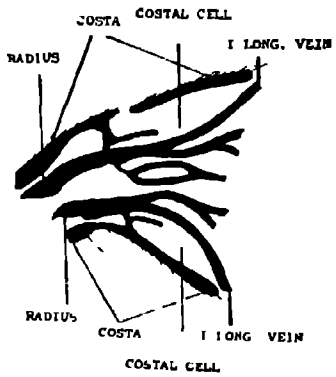

Fig. 2*d* Wing base of mirror image" duplication. Radii distinct and separate as in Fig. 1*a*

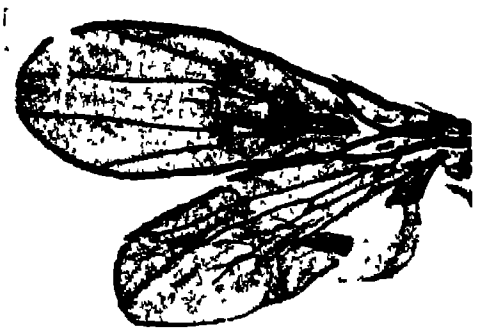

Fig. 3 Microphotograph of wing showing "triple" duplication

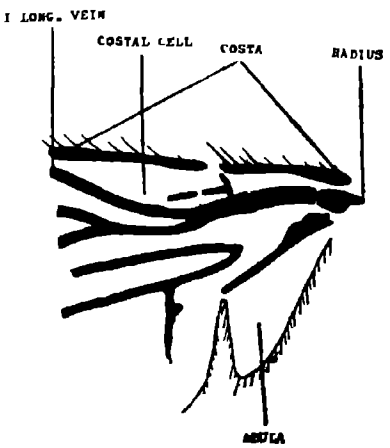

Fig. 4 Wing base– Normal

D. pseduo-obscura has shown that an alteration in the morphological characters as external appearance and shape of the egg [as in the egg mutant *Filament* (*F*)] is attended by such physiological disturbances as lowering of productivity, hatchability and fertility of eggs as compared with the normal. The foregoing data on *thorax* further lend support to idea of *manifold effects* of genes. Further analysis of known genes in Drosophila may establish the universal presence and occurrence of this phenomenon. Mullar (1922) has interpreted these effects as possibly "due to either changes of different types occurring in the same material or with changes (possibly quantitative changes similar in type) occurring in different component parts of one gene". The reason why manifold effects are not noticeble in cartain cases and are pronounced in others may in part be explained in the light of findings in developmental genetics. It has been shown there that earlier a gene comes into play in the developmental cycle of an organism, greater is the effect produced by it on other characters when it changes its normal mode of action (*i.e.*, when it mutates). Further study in this branch of biology with special reference to genes having macroscopic manifold effects will enable us to throw light on the mechanics and mechanism of gene action in development as also the nature and extent of the force or forces controlling inter- and intra-genic balances.

Summary

A complete description of the new autosomal gene in *Drosophila pseudo-obscura-thorax* (*th*) that partly overlaps wild type and affects the thorax, and its appendages, along with its linkage data is given. It is shown to be located on the third chromosome—22·4 units to the right of *orange* (*or*).

Acknowledgments

The author is indebted to Miss Rowena Lamy for the use of stocks and for general help and criticism. To Professor F. A. E. Crew his sincere thanks are due to the hospitality and sustained interest he took in the progress of this work.

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THE WEIGHT-LENGTH RELATIONSHIP OF *LABEO ROHITA* (HAMILTON) AND *CIRRHINA MRIGALA* (HAMILTON)

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In other countries much work has been done on the correlation of weight and length in the various species of fish. Spencer (1898) mentions that "a fish which has doubled its length should have increased its weight eight times". Fulton (1904) refers to the well-known law "that the volume of similarly shaped bodies of the same specific gravity[1] vary directly as the cube of the corresponding dimensions". Crozier and Hecht (1913) showed that the weight in the weak fish (*Cynoscion regalis*) increased approximately to the cube of its length. Reighard (1913) admitted that there existed a definite relation in length and weight but added that at some stage "the length increased much less rapidly than the weight" thus interfering with the relation. Clark (1925) also pointed out a definite relation in the California sardina (*Sardina caerulea*).

In India, no such work has been conducted on the different species of fish, except on "Mahseer", *Barbus* (*Tor*) *putitora* (Hamilton), for which Lacey and Cretin (1905), Skene Dhu (1906), Trevenen (1925), and Spence and Prater (1933) have advanced some formulæ. In these formulæ $1\frac{1}{2}$ length of the fish in inches [Lacey and Cretin (1905)] or $1\frac{1}{4}$ length of the fish [Trevenen (1925)], multiplied by the square of the girth in inches and divided by 1,000 gives the weight of the fish in lbs. When applied to other species of carp these formulæ do not give accurate results.

In this article, results of observations made on the weight-length relationship, in the two species of the carp, namely *Labeo rohita* (Hamilton) and *Cirrhina mrigala* (Hamilton) from the Departmental Fish Farm at Chhenawan, during the years 1938 to 1942, are given as a guide to the anglers and to the pisciculturists in other parts in India.

[1] Keys (1928) mentions that great fluctuations in specific gravity are unlikely, due to hydrostatic equilibrium that exists between the fish and its environment.

Method

Eight hundred and twenty-two fish, namely 350 Rohu, *Labeo rohita* (Hamilton) and 472 Mori, *Cirrhina mrigala* (Hamilton) were studied These fish were caught from the farm, and removed to the laboratory without much delay Measurements were immediately recorded to avoid loss of weight from evaporation The length was observed in centimetres and represented the total length from the tip of the snout to the end of the caudal fin. This was taken by placing the fish on the Fish Measuring Board[2] specially designed for the purpose The weight was recorded in 'Chhatanks' from Salter's Improved Family Scale No 50 Before the fish were weighed the surface water and mucus were removed The increase in weight of fish during the spawning season has been discounted by omitting as far as possible data on all fish with ova, when calculating the average weight The amount of undigested food was not always found sufficient to cause serious fluctuations in the average weight of fish In all these cases the observations have been recorded at different times of the year but from the same locality

The Length-Weight Relationship and its Factor

A detailed study of weights and lengths in the two species of fish at Chhenawan, showed that these two units of measure were closely related Since length is a linear measure and weight is a measure of volume, the latter would increase approximately to the cube of the length. This forms a mathematical equation,[3] namely $W = xL^3$ in which 'W' represents the weight of fish, 'L' its length and 'x' a constant, the value of which depends entirely upon the units of measure used, and on the species of fish. Such constants have been calculated at each centimetre, for each species of fish and their average has been worked out. The standard deviation and the standard error of the mean of these constants have been calculated according to the following formulæ from Davenport and Ekas (1936).

$$\theta = \sqrt{\frac{\Sigma (v - \bar{M})^2}{N}} \qquad (1)$$

where θ = Standard deviation, v = individual measure, M = mean of the above value of measures.

[2] A special board on which a scale and sliding metallic rod with a moveable pointer are fixed to record the various lengths.

[3] Clark (1928). Keys (1928) and Hile (1936) have developed this relation into the equation $W = xL^n$, where the value of both 'x' and 'n' are determined empirically After study of various formulæ the equation $W = xL^3$ was found to be sufficiently accurate.

$\Sigma(v - M)^2$ – Sum of squared deviations from the mean.
N – Number of cases.

$$SE_M = \pm \frac{\theta}{\sqrt{N}}. \quad (2)$$

where

SE_M – Standard error of the mean
θ Standard deviation.
N Number of cases

In accordance with these formulæ the following table has been formulated which gives the weight-length factor, standard deviation, and standard error for the two species of fish mentioned in this paper.

TABLE I. *Showing the weight-length factor, standard deviation and standard error for the two species of carp*

Serial No.	Species of fish	Weight-length factor	Standard deviation	Standard error
1	*Labeo rohita*	·000238	·000022891	± ·000003575
2	*Cirrhina mrigala*	·000180	·00001714	± ·000002438

The equation $W = xL^3$ can now be applied with reference to the above table. If the length of fish is known, its approximate weight can be ascertained by multiplying the cube of length of fish with the weight-length factor for that particular species of fish The average weight of fish under a given centimetre length is shown in Table II.

Conclusion

It will be seen from the tables that the weight for a given length differs in the two species considerably and so does the increment of growth for a certain increase in length. The study gives the following results:—

1. The comparative increase of weight as compared to length is more in *Labeo rohita* than *Cirrhina mrigala.*

2. If form and specific gravity remain constant, the weight of certain species of fish tends to increase approximately to the cube of its length.

3. The weight (in chhatanks) of the following species of fish can be known at a certain length (in centimetres) by multiplying the cube of the length with the weight-length factor, which is approximately as under:

(i) *Labeo rohita* . . . ·000238
(ii) *Cirrhina mrigala* .. .. ·000180

TABLE II *Showing average weight of fish at each centimetre of length for 822 fishes studied from March 1938 to December 1942*

Total length in centimetres	Species of Fish			
	Labeo rohita		*Cirrhina mrigala*	
	Average weight in chhatanks	No of fish studied	Average weight in chhatanks	No. of fish studied
13	5	2		
14	·7	2	·62	2
15	1·0	1		
16			1·0	1
17			1·0	1
18			1·0	2
19			1·0	1
21			1·5	1
28			3·5	2
30	7·0	1		
32			5·0	8
33			6·0	1
34			7·4	9
35			8·1	10
36	10·5	1	8·3	26
37			8·9	34
38			9·2	16
39			10·0	18
40	16·0	1	11·0	31
41			12·3	10
42			12·8	10
43			13·4	11
44			15·1	6
45			16·9	5
46			17·4	5
47			18·0	3
48	24·0	2	18·6	9
49	27·0	6	20·6	5
50	28·0	3	21·5	7
51	29·0	16	24·0	9
52	29·8	25		
53	33·0	33		
54	36·0	29	29·0	2
55	37·0	12	30·0	2
56	39·0	22	31·4	10
57	42·0	3	31·7	9
58	43·0	12	33 8	9
59	45·0	13	35·1	8
60	48·0	13	37·4	16
61	49·0	13	38·3	9
62	53·0	14	39·1	10
63	53·0	6	41·9	19
64	59·0	10	43·6	12
65	65·0	5	46·7	26
66	65·0	17	53·4	10
67	71·0	8	54·2	8
68			58·9	10
69	71·9	10	60·8	6
70	77·0	14	65·6	18
71	90·0	13	71·3	16
72	95·0	9	77·8	12
73	97·0	3		
74	99·8	5	81·4	5
75	102·0	7		
76	104·0	6	88·3	6
77	110·0	3	90·5	2
78	116·0	5		
80	128·0	3	99 5	4
	Total ..	350	Total ..	472

These observations are quite sufficient to show that there is a definite relationship in length and weight of the species of fish studied.

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DISEASES OF *PAN (PIPER BETLE)* IN SYLHET, ASSAM

*IV. *Rhizoctonia* Root-Rot

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CONTENTS

I. Introduction

The root-rot of *pan* (*Piper betle*, L.) due to *Rhizoctonia solani* Kuhn is very serious in Sylhet and causes heavy damage to the crop wherever it occurs. The disease attacks the roots and the stems at the ground level and causes a wilting of shoots and then the death of the entire plants.

The damage done by the disease has been found to vary from field to field. Observations made on the *pan* crop along the banks of the rivers Kachua and Juri showed that the percentage of plants killed is between 2 to 13 in different *borojes* while in the villages Machli, Mirzapore, Biabail,

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Kinnakhalpar and Haritikar, on an average 21·9 per cent. of the plants are killed annually. In a few *borojes* the loss was found even greater, the percentage of death being as high as 47·7 In a few places it is more prevalent than the *Phytophthora* root-rot, but at its worst, still it does not cause as many deaths as the latter fungus.

II. Symptoms of the Disease

The disease has been found sparingly during the hot weather and the monsoon, but gets more abundant in October and November, getting scarce in the cold weather

The fungus generally attacks the underground parts, especially the roots. It also attacks the stem at or just below the soil level. At the primary stages of attack the leaves gradually turn pale and droop and finally, in course of three to five days, the whole plant wilts and dies. When a plant is once attacked there is as a rule no recovery Examination of the stems of affected plants reveals the presence of girdling lesions at or just below the soil line. The affected tissues turn black and become markedly constricted Plants affected by roots can be easily pulled out of the ground. By removing the soil if the roots of the freshly affected plants be examined, it will be found that the roots have become reddish brown and torn to pieces

III. Inoculation Experiments

The following inoculation experiments were carried out by the pure culture of the fungus.—

(i) *Direct infection.*—

A *Pan boroj* was raised on land where *pan* had never been grown before Round about this *boroj* within a radius of 20 miles there is no *pan boroj.* Besides the parasite has not been known to attack any other host in the locality. It was divided into three plots; each plot had sixteen rows and in each of the rows six healthy *pan* setts were planted in May, 1941. The plants were inoculated in October and November, 1941 as follows:—

(*a*) the fungus was placed in the soil near the plants;

(*b*) The soil from the top was removed carefully by means of a sterilized scalpel, culture of the fungus on oat agar placed near the roots and covered up with the soil that had been previously removed;

(*c*) The fungus was placed on the part of the stem where it emerges from the soil.

The results of the inoculation experiments are summarised in Table I.

TABLE I

Summary of the inoculation experiments carried out on pan *by* Rhizoctonia solani *Kuhn*

Plots	Method of inoculation	Number of plants inoculated	Number of plants infected	Percentage of infection	Number of controls kept	Number of controls infected
I	(*a*)	72	57	79·2	24	Nil
II	(*b*)	72	59	81·9	24	Nil
III	(*c*)	72	62	86·1	24	Nil

In each case either the roots of the plants were affected or the part of the stem near the ground level became discoloured and the shoot wilted showing the typical symptoms of the disease. The same fungus was re-isolated from the dead plants and it was found to be the same as the one used to inoculate the plants

(ii) *Soil infection.*—

On land adjoining the place where experiment (i) was conducted, a second *horoj* was raised and divided into two parts one part was heavily infected with the fungus grown on paddy straw in April, 1941 and other served as control In May, 1941 after the first monsoon showers, equal number of healthy *pan* setts were planted on both the parts and kept under constant and careful observation. During this period plants of different ages wilted from time to time producing typical root rot symptoms. The results of the observations are summarised in Table II

TABLE II

Summary of the infection experiments carried out by infecting the Soil with Rhizoctonia solani *Kuhn*

Treatments	Total number of plant	Number of deaths												Total number of deaths
		June	July	Aug.	Sept.	Oct.	Nov.	Dec	Jan	Feb.	March	April	May	
(*a*) Soil infested with Rhizoctonia	120	.	.	.	2	15	27	9	.					53
(*b*) Soil not infested	120	..		..	.	..	.	..	..	.	.	.	.	..

All the dead plants were collected and examined and it was found that in all cases the death was due to *Rhizoctonia solani* Kuhn.

IV. Factors Affecting the Formation of Sclerotia in Culture

Sclerotia are the resting bodies of the fungus and as they are very important from the point of view of perpetuation and dissemination of the disease, experiments were conducted to determine the factors which favour their formation.

(i) *Media.*—

The fungus was grown for 30 days on *pan* root extract agar, oat agar, Czapeck's agar, potato dextrose agar and on Brown's synthetic agar The best growth and formation of sclerotia was noticed on oat agar, Czapeck's agar, potato dextrose agar and on *pan* extract agar. On Brown's synthetic agar mycelial growth and sclerotial formation were very poor

(ii) *Depth of the medium.*—

Depth of the medium was found to exert an influence on sclerotial formation. The fungus was grown on petri-dishes of equal sizes containing different quantities of Dox agar, 20, 40 and 70 cc Observations were recorded after 20 days and it was found that more sclerotia were formed in petri-dishes containing more medium than those containing less The best formation was in those containing 70 cc. less in 40 cc., and the least in 20 cc.

(iii) *Concentration of the medium*

Czapeck's agar of various concentrations were made and fungus grown on it for 30 days at 30° C. The results obtained are recorded in Table III.

TABLE III

Effect of concentration of the medium on sclerotial formation

Concentration	8 N	4 N	2 N	N	N/2	N/4	N/8	N/16
Formation of sclerotia	.	.	.	++++	++	+	+	+

From the data presented in Table III it will appear that the best sclerotial formation is at the normal concentration, at concentrations above the normal there is no sclerotial formation and below normal there is a decrease in the number of sclerotia formed, it being almost constant at N ½, N/8 and N/16.

(iv) *Light*

The fungus was grown on Czapeck's and oat agars and on potato dextrose agar. One set of cultures in each of these media were wrapped in paper and kept in complete darkness, the other set was kept exposed to the day

light and the darkness of the night. Practically no difference was observed in the formation of sclerotia when the cultures were kept in light or in complete darkness.

(v) *Temperature*

The fungus was grown on Czapeck's and oat agars and incubated at different temperatures for a period of 15 days The intensity of sclerotia formation observed is recorded in Table IV.

TABLE IV

Formation of sclerotia at different temperatures

Temperature	Sclerotial formation		Temperature	Sclerotial formation	
	Oat agar	Czapeck's agar		Oat agar	Czapeck's agar
10° C.		.	30° C	+++	+++
15° C	+	+	32° C	++	++
20° C	++	++	35° C	+	+
25° C	+++	++	40° C		

It will be observed from the data presented in Table IV that the best sclerotial formation takes place at 25° and 30° C. and very probably the optimum temperature for sclerotial formation lies between 25° and 30° C.

(vi) *Hydrogen-ion concentration*

The fungus was grown on modified Richards' solution (Karrer and Webb, 1920) of different pH values and its sclerotial formation noted. The observations are recorded in Table V.

TABLE V

Effect of Hydrogen-ion concentration on sclerotial formation

Hydrogen-ion concentration	Sclerotial formation	Hydrogen-ion concentration	Sclerotial formation
3·5	+++	7·6	++
3·9	+++	8·0	+
4·2	+++	8·4	+
5·0	+++	9·0	.
6·2	+++		

It will be found from the data presented in Table V that the fungus shows best sclerotial formation at 6·2 and on either side of this pH there is a

gradual fall in the formation of sclerotia but this fall is more pronounced on the alkaline side than on the acid range

(vii) *Nitrogenous, phosphatic and potassic constituents*

The effects of nitrogenous, phosphatic and potassic constituents on the formation of sclerotia were studied. As these are the principal ingredients of fertilizers it was thought that they might be of help in throwing some light on the phase of disease control by modification in the fertilizing practice. Dox agar was used for the purpose.

Nitrogen.—It was found that in the absence of sodium nitrate (the normal constituent of Dox agar) the growth is very poor and scanty and there is no formation of sclerotia When sodium nitrate is replaced by ammonium sulphate or ammonium chloride calculated to yield the same amount of nitrogen, the growth and sclerotia formation are as good as in normal solutions containing sodium nitrate.

Phosphorus and Potash.—When potassium phosphate is omitted from the medium the vigour of growth is reduced and sclerotia are altogether absent. When potassium phoshpate is replaced by potassium-di-hydrogen phosphate the sclerotial formation is as good as in the normal medium. But if potassium phosphate is replaced by magnesium phosphate the sclerotia formation is comparatively poor. This is probably due to the absence of potassium When potassium nitrate was added and there was no phosphate the growth and sclerotial formation were both found to be poor.

V. Temperature Relationship

The fungus was grown on oat and Czapeck's agar media at 15°, 20°, 25°, 28°, 30°, 35° and 40° C. temperatures. The petri-dishes used were of uniform diameter and into each was poured equal quantities of the medium in order to maintain uniformity in depth. These experiments were run in triplicate and the linear rate of growth of the fungus in both the media after five days are shown in Figs. 1 and 2.

It will be evident from the data presented in Figs. 1 and 2 that the fungus is a fast growing organism, favourable temperatures for growth being from 20° to 30° C., the optimum being at 28° C. A temperature of 10° C. and below did not permit the growth of the fungus. At 35° C. there was some growth but at 40° C. there was no growth. Petri dishes placed in the incubator at 40° C. were after 12th day placed on the laboratory table at 28-30° C. The fungus, however, failed to grow showing that prolonged exposure to that temperature had killed the fungus.

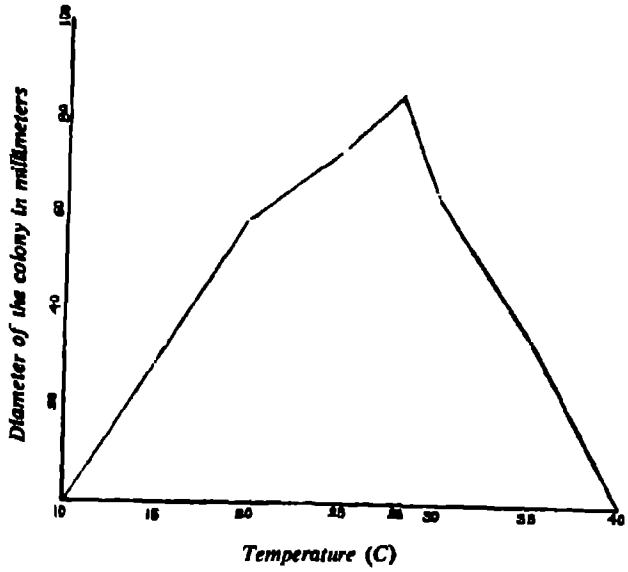

FIG. 1 Growth of *Rhizoctonia solani* in oat agar

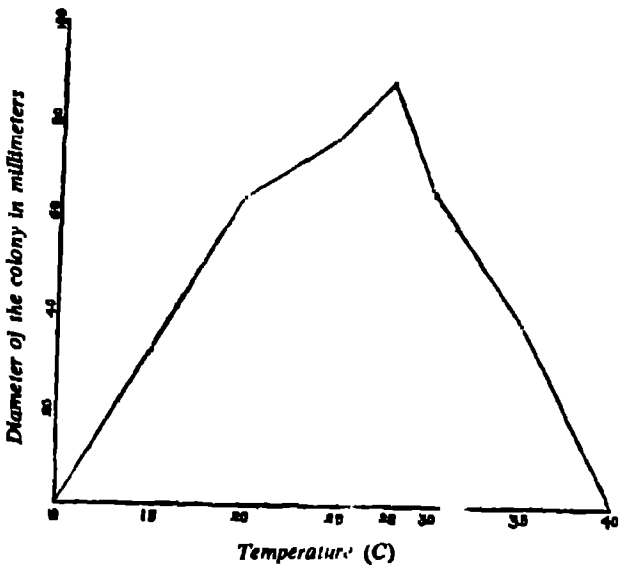

FIG. 2. Growth of *Rhizoctonia solani* in Czapeck's agar

VI. Thermal Tolerance of the Mycelium and Sclerotia

(i) *Mycelium*

A suspension of the mycelium was prepared in sterile water. Some narrow glass tubing which had been drawn into capillary forms was cut

into pieces two inches in length. Then each of these tubes was fused in the middle, thus making one inch capillary tubes sealed at one end. These tubes were then dropped into the water suspension of the fungal mycelium and exhausted under air pump. By this means the capillary tubes got filled with the suspension. They were then placed in beakers of water at various temperatures ranging from 40° to 60° C. for five minutes. Capillary tubes so treated were then broken off at the ends and the contents collected on Dox agar in petri dishes. It was observed that the fungus failed to grow when exposed at 51° C. for five minutes. This is then the thermal death point of the fungus.

(ii) *Sclerotia*

The thermal tolerance of the sclerotia was found out by immersing them for five minutes at different constant temperatures ranging from 50° to 60° C. Test tubes containing 15 cc. of sterile water were heated in a water bath to the respective temperatures Heating was done very carefully so that the temperature always remained constant and at the desired point. Sclerotia selected from cultures one month old were dropped in the test tubes containing the heated water. Immediately after the treatment cold sterile water was added and the temperature lowered down. The sclerotia were then plated on Czapeck's agar and incubated at 28°–30° C It was found that 5 minutes' exposure at 59° C. killed all the sclerotia.

It will thus appear that the sclerotia are more tolerant to heat than the mycelium.

VII Hydrogen-ion Concentration and Growth

Modified Richards' solution agar of Karrer and Webb (1920) was used; 2·5 per cent. agar was added in the solution. Petri dishes of aqual sizes were used and an equal amount of the medium poured in each. The petri dishes were inoculated in triplicate and the linear rate of growth measured from day to day. The rate of growth noticed after 5 days' growth are recorded in Table VI.

TABLE VI

Effect of Hydrogen-ion concentration on the growth of Rhizoctonia solani *Kuhn*

pH	3·2	4·0	4·6	5·2	5·6	6·2	7·0	8·0	9·1
Average colony diameter after 5 days	53·5	57·5	61·5	66·5	75·0	81·5	68·0	42·0	31·0

It will be evident from the data presented in Table VI that the fungus has a very wide range of tolerance, between 3 2 to 9 2 pH. The best growth was, however, at 6·2 This range covers all degrees of acidity that are met with in *pan horoj* soils and in ordinary arable soils. Further it has also been found that *pan boroj* soils along the river banks where the disease is also serious are definitely alkaline to reactions. Thus it is clear that a method of control of the disease by altering the pH of the soil appears out of the question

VIII Toxic Effects of Certain Chemicals

(i) *Mycelium*

The toxic effects of a few chemicals, mercuric chloride, formaldehyde, and copper sulphate on the mycelial growth of the fungus was studied. The toxic concentrations of these chemicals necessary to retard the mycelial growth of the fungus were determined by growing it on Czapeck's agar containing different percentages of the chemicals It was found as a result of this study that 0·09 per cent. mercuric chloride, 0 30 per cent. copper sulphate and 0·03 per cent. formaldehyde solutions are quite sufficient to check the growth of the fungus. Results of this study indicate that applied to the soil these chemicals at the concentrations indicated above, will kill the mycelium in the soil but it is very likely that they will not be able to kill the mycelium within the infected stems of *pan* vines that have not decayed and disintegrated. Such infected stems must be picked by hand and removed in order that this method of prevention of the disease by applying chemicals becomes efficacious.

(ii) *Sclerotia*

The toxic effects of formaldehyde and mercuric chloride on the sclerotia of the fungus was also studied by immersing the sclerotia in different concentrations of the chemicals and then growing them in agars. Sclerotia of varying sizes as are found in cultures and on the host were subjected to this treatment.

It was found that after two hours' immersion in cold formaldehyde (1 in 240) 56 per cent. of the sclerotia were still viable. A period of eight hours' immersion was necessary to kill all the sclerotia of varying sizes.

Mercuric chloride solution was found more effective than formaldehyde. It was found that sclerotia of all sizes were killed by an immersion of two and a half hours in a mercuric chloride solution of one in 834 parts of water.

IX. Viability of the Sclerotia

In order to test the viability of the sclerotia, they were picked from one month old oat agar cultures and kept in dry sand or dried in desiccators

for two days and then stored in dry sand. Their germinability which was tested from time to time, have conclusively shown that they can retain their viability even after two and half years under laboratory conditions.

The viability of the sclerotia under field conditions and at varying depths of soil was also studied. Eight glass jars each 25 cm. high were selected. Four of these jars were filled with moist soil taken from a typical *pan boroj*; the other four jars were filled with dry soil. The moisture content of the former was 25·87 and the latter 6·8 per cent.

The sclerotial bodies from 15 days' old oat agar cultures were buried in the dry soil at 5, 10 and 20 cm. depths in three of the jars. In the fourth jar the sclerotial bodies were placed on the surface. The same procedure was followed with respect to the other four jars containing moist soil from *boroj* land. The sclerotial bodies were plated out on sterilized Czapeck's agar at intervals of a month to determine whether or not they were viable.

It was found that the sclerotial bodies buried at depths of 5, 10 and 20 cm. in moist soil became sponge-like in texture and lost their vitality after 4-5 months. Some of the sclerotial bodies which were placed on the surface of moist soil grew and produced new or secondary sclerotial bodies. The mother sclerotial bodies were found to retain their viability for five months on the surface of moist soil.

The sclerotial bodies buried at different depths in dry sand lost their vitality in 6 to 7 months. On the surface of dry sand the sclerotial bodies were viable for 7 months.

X. Perpetuation and Dissemination

(i) *Perpetuation*

The fungus is essentially a soil dweller. It lives and multiplies in the soil where there is enough supply of organic matter and moisture, both of which are present in abundance in all types of *boroj* soils. The fungus may also survive in the infected plant tissues left in the soil. From a large number of these materials the fungus has been isolated. The fungus lives in the soil from year to year and from season to season by its sclerotial bodies. The fungus remains in the sclerotial stage during seasons unfavourable to its growth. When conditions are favourable the sclerotial bodies grow provided that the soil is sufficiently moist. The mycelium radiates from the sclerotial bodies and creeps on the soil until it comes in contact with its host or rotting plant materials. Under field conditions the sclerotial bodies are the chief source of infection. Under the studies on viability of the sclerotia it has been found that in moist soils the sclerotia can remain viable

for 4–5 months and can also give rise to secondary sclerotia and thus keep on living in the soil leading a saprophytic life.

(ii) *Dissemination*

Rhizoctonia solani Kuhn occurs abundantly in the *pan boroj* soils where the disease occurs In ploughing, the soil that adheres to the implements, the feet of the animals and labourers may often carry the sclerotial bodies of the fungus. The sclerotial bodies are sufficiently light to be carried from one place to another by irrigation water. These bodies may also be carried by rain water from one plot to another and also through the earth that is used by the *baruis* every year for earthing up in their *borojes*.

The fungus may also be carried through planting setts but this does not happen usually. For in selecting the setts for planting only the middle portion of the vines are chosen the lower portion lying near the ground and the top are usually discarded The fungus can only be carried through setts when they are selected from portions that lie on or near the ground and are likely to harbour the sclerotia of the fungus on their surface. It is therefore necessary that setts for planting be carefully selected

XI Control and Prevention

(a) *Control*

The control of the fungus has proved particularly difficult owing to the fact that the fungus is a soil dweller and that it is not possible to sterilize the soil and kill the parasite by keeping the crop standing in the soil. When setts are planted in a soil already infested with the parasite the disease is bound to appear under favourable conditions and under such circumstances it becomes necessary to save the standing crop.

Experiments for controlling the disease were carried out in the cultivators' *borojes* at Saiyarpur, Rattibhallabpur, Bhowanipur and also at the experimental plot at Sylhet for the last three years *Borojes* indicating very severe outbreak of the disease were selected for the treatments. They were divided into randomised blocks and given the following treatments. The treatments were:

A. Plants were left without any treatment. They served as controls.

B. The dead and the diseased plants were completely uprooted and destroyed The rest of the plants were then irrigated along the ridges with a solution of kerol (1 in 1400 parts of water). It was applied at the rate of 1 gallon of the mixture per ridge of 5 ft. in length. Every time before subsequent treatments the diseased and the dead plants were removed and destroyed.

C. Plants were simply irrigated along the ridges with a solution of kerol (1 in 1400 parts of water) without removing and destroying the dead and the diseased plants. The solution of kerol was applied at the rate of 1 gallon per ridge of 5 ft. in length

D. The dead and the diseased plants were completely uprooted and destroyed. The rest of the plants were then irrigated along the ridges with a 2:2:50 Bordeaux mixture. It was applied at the rate of 1 gallon of the mixture per ridge of 5 ft. in length. Every time before subsequent treatment the diseased and the dead plants were removed and destroyed.

E. Plants were simply irrigated along the ridges with a solution of 2:2:50 Bordeaux mixture without removing and destroying the dead and the diseased plants. The solution was applied at the rate of one gallon of the mixture per ridge of 5 ft. in length

The treatments were commenced in September and done once a month subsequently at an interval of 30 days during the months of October, November and December Thereafter no treatments were done. The number of deaths and the general appearance and vigour of the plants were noted immediately before each of the treatments and a month after the last treatment. At the time of the second treatment a decrease in the number of deaths in the treated plots was noticed and there was a distinct change in the general appearance of the plants treated

The experiments were conducted for the last three years at five different centres. For economy of space the experimental results obtained at Saiyarpur and Uttarbhag during the years 1942 and 1943 are recorded in Tables VII and VIII. At other centres exactly similar results were obtained during the years 1941, 1942 and 1943.

TABLE VII

Summary of results of experiments done for controlling the disease at Saiyarpur during the year 1942

Treatments	No. of replications	Total No. of plants	Total No. of deaths	Percentage of death
A	6	2227	325	14·6
B	6	2235	85	3·8
C	6	2232	172	7·7
D	6	2229	117	5·1
E	6	2230	191	8·5

TABLE VIII

Summary of results of experiments done for controlling the disease at Uttarbhag during the year 1943

Treatments	No of replications	Total No of plants	Total No of deaths	Percentage of death
A	6	3125	621	19·8
B	6	3115	92	2·9
C	6	2120	189	6·05
D	6	3130	176	5·6
E	6	3132	241	7·6

From the data presented in Tables VII and VIII and from the similar results obtained from experiments made at other places during the years 1941, 1942 and 1943 the following conclusions may be drawn.

(i) That the disease can be controlled to an appreciable extent by irrigating the plants along the ridges with a solution of kerol (1 in 1400 parts of water) or 2:2·50 Bordeaux mixture But kerol appears more effective than Bordeaux mixture The treatments should commence in September, before the usual time of occurrence of the disease

(ii) That in order to get satisfactory results the dead and the diseased plants should be removed and destroyed before each of the treatments, otherwise the fungus survives inside the plant tissues which the chemical solutions cannot penetrate.

(b) *Prevention.*

It has already been stated that the parasite survives in the soil and when setts are planted in infested fields the disease appears under favourable conditions. It will, therefore, become quite clear that in order to prevent the disease the following methods should be given a trial;

(i) Before planting the setts the infested soil should be sterilized. This can be done either by heat or by chemicals

(ii) After sterilizing the soil only carefully selected healthy setts should be planted. Usually setts for planting are selected from the middle portions of the vines, the lower and the top portions are discarded. It is only when lower portions are selected that there is chance of the disease being carried through setts which might have the sclerotia or the mycelium of the parasite living inside or on their surface. The lower portions and those parts of the vines lying on the soil should therefore be discarded in selecting setts for planting.

(iii) Subsequent infection through drainage water, soil and manure should be avoided

The following experiment was carried out to find the effect of soil sterilization on the prevention of the disease. A *boroj* where practically all the plants have died of the disease was selected To this an additional dose of infective material was added by introducting cultures of the fungus grown on paddy straw. The land was then divided into eight equal blocks and each block into four equal plots They were then given the following treatments and each treatment had eight replications.

A. Soils left without any treatment. They served as control

B. Soils burnt by means of rice straw, thatching grass, etc The burning operations were carried out four times thoroughly turning the soil every time The temperature of the soil upto a depth of 9 inches were taken immediately after each burning and it was found to range from 60°–65° C During burning, the temperature might have been surely more than this. As this temperature is more than the thermal tolerance of the fungus (mycelium and sclerotia) it might safely be stated that during the burning operation the heat was sufficient enough to kill the mycelium and the sclerotia of the fungus

C. Soil was treated with commercial formalin (1 part in 50 parts of water) at the rate of half a gallon of the solution per square foot

D. Soil was treated with kerol (1 part in 600 parts of water) at the rate of half a gallon of the solution per square foot.

These treatments were done in April, 1941 and in May, 1941, after a month, equal number (80 setts) of setts were planted in each of the plots. The setts were carefully selected and were free from disease Observations were made every month and the number of deaths noted. The number of deaths noted till the end of December, 1943 is recorded in Table IX

TABLE IX

Effect of sterilization of infested soils on the incidence of the disease

Treatments	Number of plants in each block	Number of deaths in each block								Total number of plants	Total number of deaths	Percentage of death
		I	II	III	IV	V	VI	VII	VIII			
A	80	16	11	17	12	13	15	10	11	640	105	16·4
B	80			2	.	3	1			640	6	0·93
C	80	2	1	..	2	4	1	3	..	640	13	2·03
D	80	2	3	2	1	..	2	2	3	640	15	2·3

It will be evident from the data presented in Table IX that infested soils can be effectively sterilized by heat, formalin or kerol and the incidence of the disease very much reduced Heat has, however, been found to be the most suitable agent for sterilizing the soil It is very simple and can be easily adopted by the illiterate grower It is true that on a field scale soil sterilization either by heat or chemicals is not practicable but in the cultivation of *pan* the method can be easily and profitably adopted *Pan* is grown on very small plots of land varying from 1/16th to 1/8th of an acre and plots of such dimensions can be easily treated with heat

During May, 1942 two *borojes* were raised at Rattibhallabpur and two at Sabia by burning the soil very thoroughly and carefully. After the burning only healthy setts were planted and every care was taken to prevent subsequent infection through manure, soil, drainage water or any other means. The *borojes* were kept under observations during the years 1942 and 1943; the number of deaths was noted and the dead plants examined microscopically. In the case of only one *boroj* a few plants were found to have died of the disease but in the case of the other three no death due to the disease could be noticed The death in the case of one *boroj* was very probably due to the fact that the soil was not thoroughly burnt

From the experiments done to control and prevent the disease and the results obtained therefrom it may be safely concluded that the disease can be effectively and economically held under check by burning the infested soil before planting and then planting only healthy setts. It is also necessary that subsequent infections be avoided The method of soil sterilization by heat as suggested in this paper is simple and easy and can be adopted by all the *pan* growers without any difficulty.

XII Summary

Root-rot of *pan* (*Piper betle*) due to *Rhizoctonia solani* Kuhn is a very serious disease in Sylhet and causes heavy damage to the crop wherever it occurs. The percentage of death of the plants varies from 2 to 47 7 annually in the different *borojes*.

The disease occurs sparingly during the hot weather and the monsoon, more abundantly during October and November and again sparingly during the winter

The symptoms of the disease have been described.

Inoculation experiments have been carried out with pure cultures of *Rhizoctonia solani* Kuhn and its pathogenecity estabilshed.

The effects of the nature of the medium, the depth of the medium, the concentration of the medium, light, temperature, hydrogen-ion concentration and the presence of nitrogenous, phosphatic and potassic constituents on the formation of sclerotia in culture have been studied and details given in the text

The favourable temperatures for the growth of the fungus are from 20° to 30°C, the optimum being at 28°C

The thermal tolerance of the mycelium and the sclerotia of the fungus was determined. It has been found that the mycelium is killed by five minutes' exposure at 51° C. and the sclerotia by an exposure of five minutes' at 59° C.

It has been found that the fungus has a wide range of hydrogen-ion concentration varying from 3·2 to 9·1 for its growth The best growth occurs at 6·2 pH.

Toxic effects of mercuric chloride, formaldehyde and copper sulphate on the growth of the mycelium and of formaldehyde and mercuric chloride on the sclerotia of the fungus were studied. The details are given in the text

The viability of the sclerotia was studied It was found that under laboratory conditions they retain their viability even after 2½ years. Under field conditions they have been found to retain their viability for 5 and 7 months on the surface of moist and dry soils respectively.

The perpetuation and dessimination of the disease were also studied. The fungus is essentially a soil dweller and survives and is desseminated through its sclerotial bodies living in the soil.

Methods of prevention and control have been studied. It has been found that the disease can be controlled by irrigating the plants along the ridges with a solution of kerol (1 in 1400 parts of water) or Bordeaux mixture (2:2:50) once a month commencing from September and ending in December

For prevention it has been found necessary to sterilize the soil before planting either by heat or chemicals. Heat, kerol and formaldehyde have been tried. Heat has given promising results and has been found to be a very effective and simple method for adoption by the cultivators In all cases it is necessary that after sterilization of the soil only healthy setts should be planted and fresh infection through infested soil or drainage-water avoided.

Acknowledgements

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A STUDY IN GASTRIC FUNCTION

Gastric Analysis of 100 Mysore Students

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Several investigations have been carried out on the response of the stomach to the test-meal in order to ascertain the variation of gastric acidity, motility and secretion in healthy subjects To cite a few of them· Bennett and Ryle[3] examined 100 healthy men in England; Baird, Campbell and Hern[1] 57 men also in England; Davis and James[5] 100 normal Americans over 60 years of age Vanzant *et al.*[14] have made a statistical study of the gastric responses of 3,746 American subjects of both sexes and of various age periods. The standards set forth by Bennett and Ryle are usually taken as normal for clinical interpretations. But these values are for Europeans The diet, general constitution and habits of different people vary The climatic conditions may also cause difference in the standard. Some attempts have been made in India to establish a standard for Indians residing in particular places Bhatia *et al*[4] experimented on 30 subjects under standard conditions. Napier and Das Gupta[13] studied the gastric analysis in 43 healthy Indians but the authors stated that the experimental conditions were far from satisfactory. Rao[13] studied the gastric response of 100 men of working class attending the hospital for eye treatment. Napier *et al.*[11] have reported the results of gastric analyses done as a routine on 209 hospital patients, who as the authors have said "cannot be regarded as normal individuals". Mangalik and co-workers[9] experimented on 75 subjects who were attending the hospital and who were considered to be normal as they had no anæmia and no signs and symptoms pertaining to gastrointestinal tract. The present paper gives an account of the investigations carried out in Mysore.

Material and Method

The material for study consisted of students and staff of the University Medical College. 80 Men and 20 women that were enjoying normal health volunteered themselves as subjects and our thanks are due to them for their willing co-operation. Their age varied from 18 to 56 years but a majority of them were students between 20 and 25 years of age. They were able to swallow the gastric tube without difficulty and retain it till the end of the

experiment. Since most of them had experimented on themselves in the practical classes the possible emotional factor influencing the results is negligible.

The subejcts were directed to fast for 12 to 14 hours overnight previous to the test. They came to the laboratory the following morning on an empty stomach. After resting for about 30 minutes they were asked to swallow Ryle's gastric tube upto the required mark An all-glass syringe (B. D. Yale) of 35 c.c. capacity was attached to the open end of the tube and with gentle suction all the resting juice was aspirated and the quantity noted. After 5 minutes' rest, the subjects were given 500 c c of freshly prepared oatmeal gruel which was at a temperature of 38° to 40° C. The temperature of the meal is of importance since it influences both the gastric secretion and motility (Gershon-Cohen *et al.*[9])

The test-meal was prepared by boiling down 2 l. of distilled water with 30 g. of oatmeal to 1½ l. It was strained through cheese cloth and cooled to 40° C. The gruel did not give a test for chloride. The freezing point of the gruel was 0·00° C. Addition of "a little salt to taste" is not an uncommon practice with some (Bennett and Ryle[3]) In the present investigation salt was purposely omitted since it would complicate the chloride estimation in fractional samples; and further it would alter the osmotic pressure of the gruel which, as has been shown by McSwiney and Spurrel,[10] has an influence on the emptying time.

At intervals of 20 minutes 15 to 20 c.c. of the gastric content was aspirated, passed through dry cheese cloth; these and the resting juice were used for the following quantitative estimations. The resting juice was qualitatively tested for starch, blood, bile and lactic acid.

Free acidity:—5 c c. of the clear filtrate was titrated against CO_2 free NaOH (0·0431 N) using 3 drops of an aqueous solution of 0·01% Tropœolin 00. Tropœolin 00 was used in preference to Topfer's reagent which according to Hori[8] gives a serious error, mainly overtitration.

Combined acidity:—The titration of the previous experiment was finished by adding 2 drops of 1·0% alcoholic solution of phenolphthalein till a permanent pink colour was got.

Total chlorides:—5 c.c. of filtrate was taken for this determination. Volhard's method was used

The data obtained have been grouped and are considered under the following categories:—

1. The resting juice and its nature.
2. The response of the stomach to the test-meal.

I The resting juice and its nature

The distribution of the cases according to the quantity of the resting juice, the maximum, the minimum and the average for the series are given in Table I. The range of variation in both sexes is great but it is less in women. The resting juice is within 50 c.c. in 69% of male and 80% of female subjects The average quantities for these two groups of subjects do not differ considerably.

TABLE I

The quantity of the resting juice

Quantity in c.c	No. of men (80)	No of women (20)
0- 9	20	3
10–19	11	2
20–29	5	2
30–39	5	4
40–49	14	5
50–59	9	3
60–69	8	1
70–79	3	
80–89	2	
90–99		
100 and above	3	
Max	135·0 c c for men	60·0 c.c for women
Min.	2·0 ,, ,,	3·0 ,, ,,
Average	36·9 ,, ,,	33·5 ,, ,,

But there is a noticeable difference in the quantity between people on vegetarian and mixed diets, as noted below:—

	Vegetarian	Mixed diet
	c.c	c.c.
Max.	110·0	62·0
Min.	4·0	3·0
Average	37·5	27·7

Too great a stress cannot be laid on this difference in view of the fact that variation in the amount of the resting juice is observed from day to day in the same individual under the same experimental conditions (Table II).

From the distribution of hydrochloric acid (Table III) it is observed that free acid is present in 64% of male and 65% of female subjects. The range of variation is considerable in both the sexes. The maximum and the average values of the acid in women are much lower than those in men.

TABLE II

Day to day variation in the resting juice of individuals in c.c.

Vegetarian		Mixed Diet	
I Day	II Day	I Day	II Day
c.c	c c.	c.c.	c.c.
35	4	62	10
9	22	10	95
7	22	25	82
2	29	7	88
58	21	40	17
60	19	18	84
9	35	30	47
15	47	65	22
40	114	2·5	38

TABLE III

Free hydrochloric acid distribution in the resting juice

Free HCl in 100 c.c of resting juice, in c c. of N/10 NaOH	No. of men (74)	No of women (20)
0·0	27	7
0– 9	10	9
10–19	11	3
20–29	13	1
30–39	7	
40–49	2	
50–59	4	
Acid Max	58·6 c c. for men	26·5 c.c for women
„ Min.	4·3 „ „	1·0 „ „
Average for people with acid	23·5 „ „	7·6 „ „
Average for all cases	14·9 „ „	5·0 „ „

For want of sufficient quantity of the resting juice total acidity could not be estimated in 8 cases. The range of variation and the averages of total and combined acids are given in Table IV. The combined acid values in

TABLE IV

Combined and total acid in the resting juice

Subjects	Combined acid in c.c.			Total acid in c.c.		
	Min.	Max.	Average	Min.	Max.	Average
Men (73)	2·2	19·8	7·8	2·2	78·4	22·9
Women (19)	2·0	19·1	7·8	2·0	42·9	12·5

90% of the subjects lie within 15 c.c.; and the average value for both men and women is the same, namely 7·8 c.c

The total chloride in the resting juice of each of 59 men and 17 women was estimated. The analysis of figures obtained is given in Table V Though the range of variation of total chlorides in the resting juice is very wide as from a minimum of 12 5 c c. to a maximum of 138 0 c.c the value is found to lie between 60 and 120 c.c in 80% of men and between 40 and 100 c c in all the cases of women.

TABLE V

Total chloride distribution in the resting juice

Total chlorides in 100 c c. of resting juice, in c.c of N/10 $AgNO_3$	No of men (59)	No of women (17)
0–9		
10–19	1	
20–29	4	
30–39	1	
40–49	3	3
50–59	1	1
60–69	6	3
70–79	9	5
80–89	8	2
90–99	9	3
100–109	8	
110–119	4	
120–129	4	
130–139	1	
Max of total chlorides	138·0 c c for men	97·2 c c for women
Min. ,,	12·5 ,, ,,	41·0 ,, ,,
Average ,,	81·2 ,, ,,	71·5 ,, ,,

The qualitative tests of the resting juice revealed the absence of starch and lactic acid in all the samples. Bile was present in 42% and mucus in 60% of men; among women bile and mucus were present in 68% of cases

2. The response of the stomach to oatmeal gruel

The maximum, the minimum, the mean and its standard deviation of the free HCl response at various fractional intervals for all the 100 cases are summarised in Table VI Charts I and II show the limits of variation in free acid response at various fractional intervals in 80% of cases of men and women respectively.

After the test-meal the first fractional sample showed the free acid value to be invariably lower than that of the resting juice owing to, perhaps,

dilution by the gruel. The free acid in the subsequent samples gradually increased upto a maximum and then decreased, a secondary rise in free acid

TABLE VI

The maximum, the minimum, the mean and its standard deviation of free hydrochloric acid response at various fractional intervals

Sample at the end of	Men				Women			
	Max. in c.c	Min. in c.c	Mean in c.c	Standard deviation of the mean	Max. in c c	Min in c c	Mean in c.c	Standard deviation of the mean
Fasting	58·6	0·0	15·4	±14·3	26·5	0·0	5·5	± 4·4
20 min	23·3	0·0	8·1	± 5·9	15·2	0·0	5·8	± 3·8
40 ,,	46·7	0·0	19·3	± 9·9	41·6	1·3	13·5	± 9·5
60 ,,	69·8	0·0	26·5	±14·9	44·1	0·0	15·4	±11·5
80 ,,	79·3	0·0	26·6	±15·6	48·2	9·5	19·0	+11·7
100 ,,	78·4	0·0	28·4	±17·1	40·9	13·6	21·1	± 9·8
120 ,,	79·6	0·0	32·5	±16 5	22·0	11·2	17·0	± 3·5
140 ,,	94·0	0·0	23·1	±10·8	30·1	8·6	18·6	±10·2
160 ,,	27·2	13·0	20·3	± 8·4				

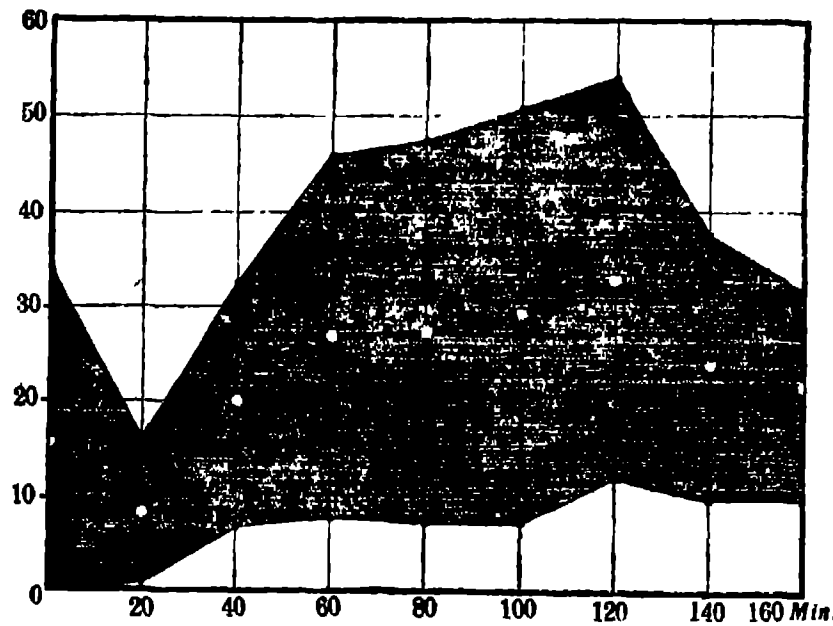

Chart I. To show the range of variation in free HCl response in 80% of male subjects at different intervals of fractional test-meal. The range is mean ±1·25 0.

O——O Curve of mean free acidity

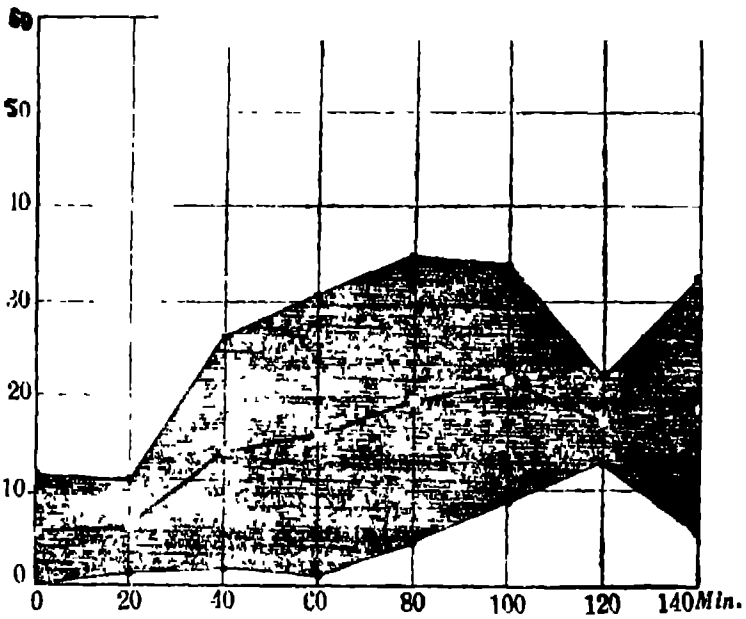

Chart II. To show the range of variation in free HCl response in 80% of female subjects at different intervals of fractional test-meal The range is mean ±1·25 0.

O——O Curve of mean free acidity

being observed in 20% of male and 5% of female subjects The distribution, the range of variation and the average of free acid maximum for men and women are given in Table VII. The average value of free acid is 36·4 c.c for men and 22·8 c.c for women

TABLE VII

Free hydrochloric acid maximum in fractional samples

Free HCl in 100 c c of juice, in c.c. of N/10 NaOH	No of men (80)	No. of women (20)
No free HCl	0	0
0–9	1	3
10–19	7	6
20–29	24	7
30–39	17	1
40–49	15	3
50–59	11	.
60–69	1	.
70–79	1	..
80–89	2	..
90–99	1	..
Max.	94·0 c.c. for men	48·2 c.c. for women
Min.	8·6 ,, ,,	4·7 ,, ,,
Average of the Maxima	36·4 ,, ,,	22·8 ,, ,,

The classification of free HCl into various groups (according to the arbitrary standards used by Napier *et al*[11]) given in Table VIII. shows the following features. Achlorhydria is totally absent in the present series 93·8% of men are in normal zone of acidity, the rest being constituted of hypochlorhydrics and hyperchlorhydrics. The normal zone cases are distributed in the three subgroups of low, medium and high normal acidities in the ratio of 1 : 2 : 1 Among women, 75% of subjects are in normal zone and these are distributed in the three sub-groups in the ratio of 9 : 5 : 1 The remaining 25% are hypochlorhydrics, there being no hyperchlorhydric Thus, there is a preponderance of hypochlorhydric and low normal acid cases, accounting for 70% of the total women subjects.

TABLE VIII

Classification of free acid into various groups

	Hypochlor-hydria (below 10 c.c)	Normal zone			Hyperchlor-hydria (above 65 c.c.)	Achlor-hydria (0 c c)
		Low (10–25 c.c.)	Medium (25–45 c.c.)	High (45–65 c.c)		
No of men	1	17	39	19	4	0
Percentage .	1·2	21·2	48·8	23·8	5·0	0
No. of women	5	9	5	1	0	0
Percentage .	25·0	45·0	25·0	5·0	0	0

The mean acidity curve of women (Chart III) runs almost parallel to and at a definitely lower level than that of men, showing that mean free acid values at various fractional intervals are less in women than in men

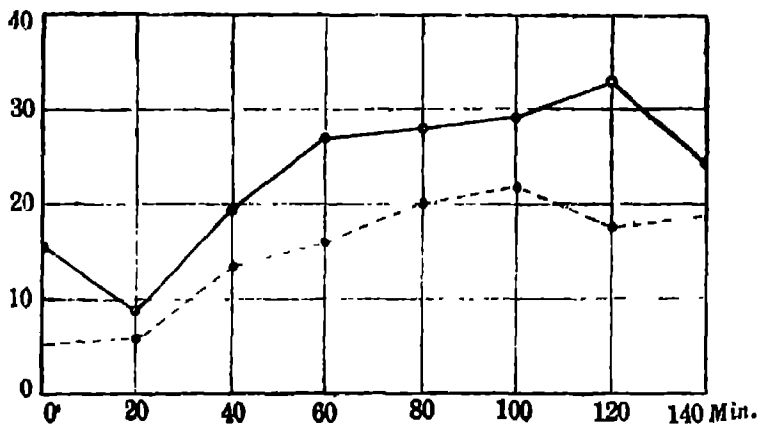

Chart III. To show the mean free acidity curves of males and females

Mean free acidity in males.
Mean free acidity in females.

The distribution of cases according to the time at which the free acid becomes a maximum during a fractional test-meal is shown in Table IX. The average time is 77 minutes for men and 67 minutes for women. The free acid reaches a maximum between 60 and 100 minutes in 72·5% of men, whereas in 75% of women it is between 40 and 80 minutes.

TABLE IX

Time at which free hydrochloric acid maximum is reached

	No of men (80)	No of women (20)
At the end of 20 minutes	1	1
,, 40 ,,	12	6
,, 60 ,,	20	5
,, 80 ,,	22	4
,, 100 ,,	16	2
,, 120 ,,	7	1
,, 140 ,,	2	1
Average time at which free acid reaches max	77 minutes	67 minutes

The curve of total acidity in every case runs parallel to and at a higher level than that of the free HCl. The distribution, the range and the average of combined acid is given in Table X The average value for men and women does not differ much In majority of cases in both sexes the highest combined acid value is attained in the fraction that shows the free acid maximum.

TABLE X

Combined acid maximum

Combined acid in 100 c c of gastric juice, in c c of N/10 NaOH	No of men (80)	No of women (20)
Less than 4 c c		3
4–5·9	1	1
6–7·9	7	1
8–9·9	14	3
10–11·9	25	4
12–13·9	20	5
14–15·9	9	2
16–17·9	4	1
Combined acid Max	17·4 c c for men	17·2 c c for women
,, Min	5·2 ,, ,,	3·8 ,, ,,
,, average	11·5 ,, ,,	10·4 ,, ,,

The distribution of maximum total chloride is given in Table XI. In 80% of the cases the maximum is reached by the end of gastric digestion of the test-meal and in 20% a little earlier.

TABLE XI

Total chlorides maximum

Total chlorides in 100 c.c. of gastric juice, in c c. of N/10 $AgNO_3$	No. of men (80)	No of women (20)
20- 29		2
30- 39		3
40- 49	5	1
50- 59	5	4
60- 69	4	6
70- 79	18	1
80- 89	12	1
90- 99	18	2
100-109	8	
110-119	5	
120-129	3	
130-139	2	
Chlorides Max	138·6 c c. for men	90·0 c c for women
,, Min	40·0 ,, ,,	20·0 ,, ,,
,, average	85·5 ,, ,,	57·1 ,, ,,

From Table XII which gives the emptying time, it is observed that in 82·5% of men the stomach empties in 80 to 120 minutes. The average emptying time is 105 minutes excluding the three cases in which the stomach was not empty even at the end of 180 minutes. In 60% of women subjects the stomach emptied in 60 to 100 minutes and in 20% of cases the emptying time is as early as 40 minutes, the average being 78 minutes

TABLE XII

Emptying time

	No. of men (79)	No of women (20)
At the end of 40 minutes		4
,, 60 ,,	2	8
,, 80 ,,	13	2
,, 100 ,,	33	2
,, 120 ,,	20	1
,, 140 ,,	8	2
,, 160 ,,		1
,, 180 ,, and beyond	3	
Average emptying time	105 minutes	78 minutes

Comment

Certain physiological factors like sex, dietetic habit and age which are likely to influence the gastric response are considered here.

The free hydrochloric acid secretion in women is definitely lower than in men as judged from the maxima and the mean of the free acid both in

the resting juice and in the fractional samples and from the relative preponderance of low normal and hypochlorhydric cases among women. But the motility of the stomach is greater in women.

In order to find out the existence of any possible difference in the gastric response to a test meal between people on vegetarian and mixed diets one dozen cases from each group, corresponding in age, stature and habit of life (active or sedentary) were selected. The chief features of their responses to gruel meal are summarised in Table XIII. The free acid responses of these two groups as shown by the composite curves (Chart IV) are very similar. Though the maximum acid values are somewhat varied,

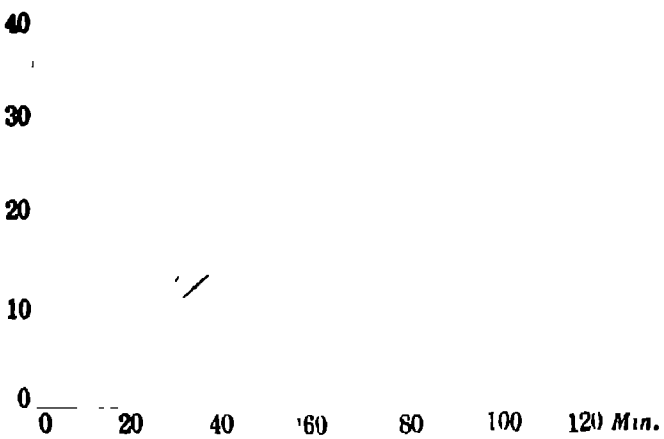

Chart IV To show the mean free acidity curves of people on vegetarian and mixed diets

Mean free acidity, mixed diet
Mean free acidity, vegetarian

the minimum and the mean of these two groups do not differ much. Time of acid peak is the same. Emptying time differs very little. Thus, there is no difference in the gastric response of people on vegetarian and mixed diets.

TABLE XIII

	Vegetarian Diet	Mixed Diet
Free acid max . . .	53·9 c.c	69·8 c.c
,, min .	12·9 ,,	19·0 ,,
Average of free acid max. ..	32·0 ,,	37·4 ,,
Time at which free acid becomes max	82 minutes	82 minutes
Emptying time . .	95 ,,	100 ,,

That the age is a factor that influences gastric secretion has been amply made out by Vanzant *et al*[16] But we are not in a position to draw any such inference as the greater number of our subjects belonged to only one age period, *viz.*, 20 to 25 years

A comparison of the studies on the gastric function that have been made in several parts of India (*vide supra*) to elucidate any difference, has to be made with caution. First, there is no uniformity regarding the test-meal Oatmeal gruel was used in the Calcutta and Assam series and alcohol in the Bengal series by Napier and co-workers. Rao's study was based on gruel meal. Mangalik *et al.* used gruel in a few cases and alcohol in a large number of cases. Secondly, regarding the standard as to what constitutes a normal range for free HCl response in a population, different investigators have accepted different criteria Following the arbitrary standard of Bennett and Ryle, Rao considers the response in 80% of his subjects to constitute the normal range; Napier and co-workers give the range as mean ± its standard deviation and it is not clear what percentage of cases lies within this range; Mangalik *et al.* take the response in 88% of cases as normal range. Thirdly, it may be noted that of the two series of observations made by Napier *et al* on Bengal subjects, only one made on normal people could be used for comparison. In the other (Gastric acidity in health and disease in India) almost all subjects were suffering from one of the conditions like anæmia, gastric and intestinal disorders, Kala-azar, malaria, leukæmia, etc. Curiously the authors make use of the results of this series for purposes of inference as though they were normal; and Mangalik *et al* use it for comparing their results obtained on normal people.

In the comparative table (Table XIV) giving the essential features of the results of several investigators only figures pertaining to gruel meal have been made use of

Resting juice (Table XIV). The average quantity in Mysore subjects is slightly greater than in those of other provinces in India. The mean free acidity of Andhra and Assam people is markedly higher than in others. The relative frequency with which bile appears in the resting juice of Mysoreans and the English is nearly twice that of the Andhra and Bombay subjects. The resting juice in the English does not significantly differ from that of the Indians but the quantity is higher.

TABLE XIV

Comparative statement

		Bennett and Ryle	Napier and Das Gupta	Napier and Das Gupta	Napier and Das Gupta	Bhatia *et al*	Rao	Mangalik *et al*	Mangalik *et al*	Srikantia *et al*	Srikantia *et al*
	Number of cases investigated	100	14	15	14	30	100	29	75	80	20
	Sex	M	M	M	F	M	M		M & F	M	F
	Age in years		20–40		13–50				18–70	20–25 mostly	18–30
	Occupation	Students	Menial staff	Plantation coolies		Students mostly	Patients	Patients		Mostly students	
	Method employed	Rehfuss									
		Boas meal					Boas meal		Alcohol	Boas meal	
Resting juice	Average volume	54·0	25·5			28·5	30·0		30·0	36·9	33·5
	Range in c.c	10–150	12–46	0–50	0–50	2·7–110	2–95			2–135	3–60
	Mean free acid in c.c 0·1N NaOH	11·5*	16 0	25·0	14·0	14·2	23·0*	12·4	14 2	14·9	5·0
	Percentage of cases with-free-HCl	54·0* (not-less than)	84·0	83 0	64·0	73·0			52·0	63·5	65·0
	Percentage of cases with bile	40				20	22			42	68
Gastric response to gruel meal	Max. free HCl in c.c for the whole series	104·0	90·0	110·0			86·0†	96·0		94·0	48·2
	Time of "acid peak" in min for the whole series	120	75	75			120	45		140	80
	Highest value in the mean acidity curve in c.c.	27·0	42·0	34·2			42·0	33·4		32·5	21·1
	Initial range of free HCl in 80 per cent of cases in c.c	0–23·0	0–35·1	0–35·5			0–50·0		0–58 0	0–33 3	0–11·0
	Max. free HCl in 80 per cent of cases in c.c	47·0	68·4	65·2			61·0			53·1	33·6
	Average time of "acid peak" for the series and time of "acid peak" in majority of cases in min.	77·5*	90 0				67·0*	90·0		77·0	67·0
		60–105	75–105	60–75			30–75			60–100	40–80
	Percentage of cases with achlorhydria	4·0	2·3				3·0		6·0	0·0	0·0
	Average emptying time of the series	114					68			105	78
	Range in majority of cases in min.	90–150					45–75			80–120	40–100

* These figures are calculated from tables given by the authors † Value as given in the text; as per graph 2, it is 108

M = male; F = female

The response

The response to gruel meal also shows comparable features pertaining to secretion of free acid and rate of emptying of the stomach. The response in 80% of cases only is considered here except in the case of Bengal subjects in whom the range of acid response is given by mean ± its standard deviation (Table XV).

TABLE XV

The subjects	Range of initial acidity in c c	Max free HCl in c c	Time of "acid peak" in min	Highest value in the mean acidity curve in c c	Emptying time in minutes
Bengal	0–35·1	68·4	75–105	42·0	
Andhra	0–50·0	61·0	30– 75	42·0	45– 75
The U P	0–58·0		90·0	33·4	
Mysore	0–33·0	53·1	60–100	32·5	80–120
England	0–23·0	47·0	60–105	27·0*	90–150

* Taken from the curve given by Bennett and Ryle [3] Mangalik *et al.*[9] report that this has not been worked out by Bennett and Ryle. Presumably they had no access to the original

An examination of the above table clearly shows that the free acid response of subjects of Mysore and the U P (which are nearly similar as judged by mean acidity) is less than that of the people of Andhra and Bengal. The response in Andhra subjects occurs earlier than in others. Free acid response of Indians is greater than that of the English. The motility of the stomach is relatively great in Andhra subjects, less in Mysoreans and still less in the English.

Achlorhydria

We find none of our subjects to have achlorhydria Achlorhydria is observed by Napier *et al.*[12] in 2·3% in one and about 6% in another series,[11] by Rao[13] in 3%, and by Mangalik and co-workers[9] in 7 7%. For Western subjects percentages varying from 4 to 23 have been reported. Even in one and the same people different figures of incidence have been observed. Whilst Bell[8] has noted 13·8% of cases to be achlorhydric among the English, Bennett and Ryle[3] have observed it in only 4%. Similar is the record for Americans as observed by Vanzant *et al*[14] Even a casual survey of the literature reveals that there is no agreement of opinion as to the frequency of this defect among otherwise normal persons. Besides, factors like race, conditions of nutrition and climate which may bring about a difference in the incidence of achlorhydria, the variations noted above may be attributable to two causes. First, the criterion for achlorhydria has not been the same. In recent times, more or less the universally accepted criterion is the absence

of free acid response to histamine injection The findings of Bennett and Ryle,[3] of Rao[13] and presumably of Napier and Das Gupta[12] are not based on this criterion. Secondly, there is no clear analysis of achlorhydric cases with reference either to age periods or sex; hence the variations in the incidence noted above, are probably due to inclusion of varying number of cases of different age groups and of different sexes in each series For Vanzant and co-workers[14] have shown that the incidence bears a straight line correlation with age; further they have made out that this defect is not present below the age of 25 and that the incidence increases upto 60 to 65 years and thereafter falls; they have also observed that at all ages women show a greater tendency to achlorhydria than men In the light of this, it is not surprising to find the absence of achlorhydria in our series since more than 80% of subjects belong to an age period of 20 to 25 years.

Exercise and Gastric Response

The effect of physical exercise on gastric secretion was investigated in only two of our subjects whose gastric response to gruel meal was normal Gastric analysis was done after strenuous exercise. There was the absence of free acid in all fractional samples which showed increasing amount of chlorides While confirming the observation of Hellebrandt *et al.*[7] that exercise depresses the acid secretion, one is forcibly reminded of the importance of avoiding physical strain before gastric analysis.

Our thanks are due to Mr M V. Lakshminaranaiya who helped us in some of the analysis.

Summary

1. Fractional gastric analysis, using Boas meal, has been done in 100 normal Mysoreans (80 men and 20 women) between 20 and 25 years of age.

2. Free acid, total acid and total chlorides have been estimated in the resting juice and in all fractional samples. Emptying time is noted.

3. A difference in the gastric response in the two sexes, observed by other investigators, has been confirmed.

4. No appreciable difference is observed in the gastric responses of people on vegetarian and mixed diets.

5. There was no case of achlorhydria in our series.

6. Depressant action of physical strain before gastric analysis on acid secretion has been pointed out.

7. Standard reference charts for men and women, to show the range of variation in maximum HCl response throughout the gastric cycle in 80% of cases are given.

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